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<b>(21) International Application Number:</b> PCT/US97/03461 <b>(22) International Filing Date:</b> 14 March 1997 (14.03.97) <b>(30) Priority Data:</b> 08/615,944 14 March 1996 (14.03.96) US <b>(60) Parent Application or Grant</b> <b>(63) Related by Continuation</b> US 08/615,944 (CIP) Filed on 14 March 1996 (14.03.96) <b>(71) Applicant (for all designated States except US):</b> WASHINGTON UNIVERSITY [US/US]; One Brookings Drive, St. Louis, MO 63130 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> JOHNSON, Eugene, M., Jr. [US/US]; 13233 Arnot Drive, St. Louis, MO 63146 (US). MILBRANDT, Jeffrey, D. [US/US]; 75 Aberdeen Place, St. Louis, MO 63105 (US). KOTZBAUER, Paul, T. [US/US]; 239 Crandon Drive, St. Louis, MO 63105 (US).		<b>LAMPE, Patricia, A. [US/US];</b> 10323 Grant Forest Lane, St. Louis, MO 63123 (US). <b>(74) Agents:</b> HOLLAND, Donald, R. et al.; Howell & Haferkamp, L.C., Suite 1400, 7733 Forsyth Boulevard, St. Louis, MO 63105-1817 (US). <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PERSEPHIN AND RELATED GROWTH FACTORS <b>(57) Abstract</b> <p>A novel growth factor, persephin, which belongs to the GDNF/neurturin family of growth factors, is disclosed. The mouse and rat amino acid sequences have been identified. Mouse and rat persephin genomic DNA sequences have been cloned and sequenced and the respective cDNA sequences identified. In addition, methods for treating degenerative conditions using persephin, methods for detecting persephin gene alterations and methods for detecting and monitoring patient levels of persephin are provided. Methods for identifying additional members of the persephin-neurturin-GDNF family of growth factors are also provided.</p>		

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PERSEPHIN AND RELATED GROWTH FACTORS

Reference to Government Grant

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5 Background of the Invention

(1) Field of the Invention

This invention relates generally to trophic or growth factors and, more particularly, to novel growth factors of the neurturin-GDNF family of growth factors.

10 (2) Description of the Related Art

The development and maintenance of tissues in complex organisms requires precise control over the processes of cell proliferation, differentiation, survival and function. A major mechanism whereby these  
15 processes are controlled is through the actions of polypeptides known as "growth factors". These structurally diverse molecules act through specific cell surface receptors to produce these actions.

Growth factors, termed "neurotrophic factors" promote the differentiation, growth and survival of  
20 neurons and reside in the nervous system or in innervated tissues. Nerve growth factor (NGF) was the first neurotrophic factor to be identified and characterized (Levi-Montalcini et al., *J. Exp. Zool.* 116:321, 1951  
25 which is incorporated by reference). NGF exists as a non-covalently bound homodimer that promotes the survival and growth of sympathetic, neural crest-derived sensory, and basal forebrain cholinergic neurons. In sympathetic neurons this substance produces neurite outgrowth in  
30 *vitro* and increased axonal and dendritic growth *in vivo*. (See Levi-Montalcini and Booker, *Proc Nat'l Acad Sci* 46:384-391, 1960; Johnson et al. *Science* 210: 916-918, 1980; Crowley et al., *Cell* 76:1001-12, 1994 which are

incorporated by reference). NGF has effects on cognition and neuronal plasticity, and can promote the survival of neurons that have suffered damage due to a variety of mechanical, chemical, viral, and immunological insults (Snider and Johnson, *Ann Neurol* 26:489-506, 1989; Hefti, *J Neurobiol* 25:1418-35, 1994 which are incorporated by reference). NGF also is known to extensively interact with the endocrine system and in immune and inflammatory processes. (Reviewed in Scully and Otten, *Cell Biol Int* 19:459-469, 1995; Otten and Gadiant, *Int. J. Devl Neurosci* 13:147-151, 1995 which are incorporated by reference). For example, NGF promotes the survival of mast cells. (Horigome et al. *J Biol Chem* 269:2695-2707, 1994 which is incorporated by reference).

In recent years it has become apparent that growth factors fall into classes, i.e. families or superfamilies based upon the similarities in their amino acid sequences. These families include, for example, the fibroblast growth factor family, the neurotrophin family and the transforming growth factor-beta (TGF- $\beta$ ) family. As an example of family member sequence similarities, TGF- $\beta$  family members have 7 canonical framework cysteine residues which identify members of this superfamily.

NGF is the prototype of such a family of growth factors. Brain-derived neurotrophic factor (BDNF), the second member of this family to be discovered, was shown to be related to NGF by virtue of the conservation of all six cysteines that form the three internal disulfides of the NGF monomer (Barde, *Prog Growth Factor Res* 2:237-248, 1990 and Liebrock et al. *Nature* 341:149-152, 1989 which are incorporated by reference). By utilizing the information provided by BDNF of the highly conserved portions of two factors, additional members (NT-3, NT-4/5) of this neurotrophin family were rapidly found by several groups (Klein, *FASEB J* 8:738-44, 1994 which is incorporated by reference).



Neurotrophic factors structurally unrelated to NGF have been recently identified. These include factors originally isolated based upon a "neurotrophic action" such as ciliary neurotrophic factor (CNTF) (Lin et al.,  
5 Science 246:1023-5, 1989 which is incorporated by reference) along with others originally isolated as a result of non-neuronal activities (e.g. fibroblast growth factors (Cheng and Mattson Neuron 1:1031-41, 1991 which is  
10 486:396-398, 1989 which is incorporated by reference) leukemia inhibitory factor (Kotzbauer et al, Neuron 12:763-773, 1994 which is incorporated by reference).

Glial-derived neurotrophic factor (GDNF), is one such neurotrophic factor structurally unrelated to NGF.  
15 GDNF was, thus, a unique factor, which, up until now, was not known to be a member of any subfamily of factors. The discovery, purification and cloning of GDNF resulted from a search for factors crucial to the survival of midbrain dopaminergic neurons, which degenerate in  
20 Parkinson's disease. GDNF was purified from rat B49 glial cell conditioned media (Lin et al., Science 260:1130-2, 1993 which is incorporated by reference). Sequence analysis revealed it to be a distant member of the TGF- $\beta$  superfamily of growth factors, having  
25 approximately 20% identity based primarily on the characteristic alignment of the 7 canonical framework cysteine residues (Lin et al., Science 260:1130-2, 1993 which is incorporated by reference). Thus, GDNF could possibly have represented a new subfamily within the TGF- $\beta$   
30 superfamily.

Recombinant GDNF produced in bacteria specifically promotes the survival and morphological differentiation of dopaminergic neurons (Lin et al., Science 260:1130-2, 1993); Tomac et al., Nature 373:335-9, 1995; Beck et al.,  
35 Nature 373:339-41, 1995 and Ebendal et al., J Neurosci Res 40:276-84, 1995 which are incorporated by reference)

and motor neurons (Henderson et al., *Science* 266:1062-4, 1994; Yan et al., *Nature* 373:341-4, 1995; and Oppenheim et al., *Nature* 373:344-6, 1995 which are incorporated by reference). Overall, GDNF was a more potent factor for promoting the survival of motor neurons than the other factors, and it was the only factor that prevented neuronal atrophy in response to these lesions, thereby positioning it as a promising therapeutic agent for motor neuron diseases.

It is now generally believed that neurotrophic factors regulate many aspects of neuronal function, including survival and development in fetal life, and structural integrity and plasticity in adulthood. Since both acute nervous system injuries as well as chronic neurodegenerative diseases are characterized by structural damage and, possibly, by disease-induced apoptosis, it is likely that neurotrophic factors play some role in these afflictions. Indeed, a considerable body of evidence suggests that neurotrophic factors may be valuable therapeutic agents for treatment of these neurodegenerative conditions, which are perhaps the most socially and economically destructive diseases now afflicting our society. Nevertheless, because different neurotrophic factors can potentially act preferentially through different receptors and on different neuronal or non-neuronal cell types, there remains a continuing need for the identification of new members of neurotrophic factor families for use in the diagnosis and treatment of a variety of acute and chronic diseases of the nervous system.

#### Summary of the Invention:

Briefly, therefore, the present invention is directed to the identification and isolation of substantially purified factors that promote the survival and growth of neurons as well as non-neuronal cells. Accordingly, the inventors herein have succeeded in

discovering novel protein growth factors belonging to a family of growth factors for which GDNF was the first known member. The first such newly discovered family member was neurturin and this is the subject of copending application serial Number 08/519,777. Based upon the sequence of GDNF and neurturin the inventors herein have discovered another member of the GDNF-Neurturin family of growth factors referenced herein as persephin (PSP). This growth factor is believed to show at least 85% sequence identity among homologous sequences from different mammalian species although sequence homology may be as low as 65% in non-mammalian species such as avian species. Persephin proteins identified herein include mouse sequences as set forth in SEQ ID NOS:79, 80 and 81 (Figure 11; amino acid residues 52 through 140, 47 through 142, and 9 through 142, respectively) and rat sequences as set forth in SEQ ID NOS:82 and 83 (Figure 14; amino acid residues 1 through 89 and 1 through 91, respectively). In addition, human persephin is identified by virtue of its having at least 85% sequence homology with its ortholog, mature mouse persephin, along with the identification of certain conserved amino acid residues contained within human persephin as shown in Figure 15. Thus, it is believed that human persephin will have 28 amino acids in the aligned sequence between the first and seventh canonical framework cysteine residues as set forth in Figure 15 with residues numbered from the N-terminal end of the family member aligned sequence being (1) Cys, (3) Leu, (10) Val, (13) Leu, (14) Gly, (15) Leu, (16) Gly, (17) Tyr, (21) Glu, (25) Phe, (26) Arg, (27) Tyr, (28) Cys, (30) Gly, (32) Cys, (44) Leu, (47) Leu, (58) Cys, (59) Cys (61) Pro, (66) Asp, (69) Phe, (70) Leu, (71) Asp, (83) Ser, (84) Ala, (87) Cys, and (89) Cys.

Persephin has been identified and obtained by a method based upon the conserved regions of the GDNF-

Neurturin family discovered by the inventors herein. Accordingly, a new method has been devised that utilizes degenerate primers constructed from the sequences of these conserved regions for use in the polymerase chain  
5 reaction procedure. By utilizing this method the mouse and rat orthologs of the new family member, persephin, have been identified and obtained.

The present invention thus provides both amino acid sequences and nucleotide sequences that encode mouse  
10 and rat persephin as set forth in the amino acid sequences of SEQ ID NOS:79-83 and nucleotide sequences of SEQ ID NOS:84 and 85. Because of the close homology between the mouse and rat sequences (95% sequence identity), it is believed that the human persephin  
15 sequence will show a high sequence homology to the mouse and rat sequences.

Expression vectors and stably transformed cells are also within the scope of this invention. The transformed cells can be used in a method for producing  
20 persephin.

In another embodiment, the present invention provides a method for preventing or treating neuronal degeneration comprising administering to a patient in need thereof a therapeutically effective amount of  
25 persephin. A patient may also be treated by implanting transformed cells which express persephin or a DNA sequence which encodes persephin into a patient, or cells cultured and expanded by growth in persephin.

The present invention also provides compositions  
30 and methods for detecting persephin. One method is based upon persephin antibodies and other methods are based upon detecting mRNA or cDNA or genomic DNA encoding persephin using recombinant DNA techniques.

Among the several advantages found to be achieved  
35 by the present invention, therefore, may be noted the provision of a new growth factor, persephin, for use in

preventing the atrophy, degeneration or death of certain cells, in particular neurons; the provision of human persephin by making available the specific sequences of murine and rat persephin from which the human sequence  
5 can be identified and obtained; the provision of other members of the neurturin-persephin-GDNF family of growth factors by making available new methods capable of obtaining other family members; the provision of methods for obtaining persephin by recombinant techniques; the  
10 provision of methods for preventing or treating diseases producing cellular degeneration and, particularly neuronal degeneration; the provision of methods that can detect and monitor persephin levels in a patient; and the provision of methods that can detect alterations in the  
15 persephin gene.

#### Brief Description of the Drawings

Figure 1 illustrates the purification scheme for preparing neurturin from CHO cells;

Figure 2 illustrates the characterization of  
20 fractions eluted from Mono S column in purifying neurturin showing (a) electrophoresis of each fraction on a SDS-polyacrylamide gel and visualization of the proteins by silver stain and (b) the neurotrophic activity present in each fraction in the superior  
25 cervical ganglion survival assay;

Figure 3 illustrates the ability of neurturin to maintain survival of superior cervical ganglionic cells in culture showing (a) positive control cells maintained with nerve growth factor (NGF) (b) negative control cells  
30 treated with anti-NGF antibodies showing diminished survival and (c) cells treated with anti-NGF and neurturin (approximately 3 ng/ml) showing survival of neurons;

Figure 4 illustrates the concentration-response  
35 effect of neurturin in the superior cervical ganglion survival assay;

Figure 5 illustrates the homology of the amino acid sequences for the mature growth factors, human neurturin (hNTN), mouse neurturin (mNTN), rat GDNF (rGDNF), mouse GDNF (mGDNF) and human GDNF (hGDNF) with 5 identical amino acid residues enclosed in boxes;

Figure 6 illustrates the tissue distribution of neurturin mRNA and the mRNA for GDNF using RT/PCR analysis on RNA samples obtained from embryonic day 21 (E21) and adult rats;

10 Figure 7 illustrates the cDNA and encoded amino acid sequence of human pre-pro neurturin (SEQ ID NO:11) showing the pre- region from nucleic acid 1 through 57 (SEQ ID NO:17), the pro- region from nucleic acid 58 through 285 (SEQ ID NO:20), human neurturin from nucleic 15 acid 286 through 591 (SEQ ID NO:9) and the splice site between nucleic acids 169 and 170 which defines the coding sequence portion of two exons from nucleic acids 1 through 169 (SEQ ID NO:27) and 170 through 594 (SEQ ID NO:28);

20 Figure 8 illustrates the cDNA and encoded amino acid sequence of mouse pre-pro neurturin (SEQ ID NO:12) showing the pre- region from nucleic acid 1 through 57 (SEQ ID NO:18), the pro- region from nucleic acid 58 through 285 (SEQ ID NO:21), mouse neurturin from nucleic 25 acid 286 through 585 (SEQ ID NO:10) and the splice site between nucleic acids 169 and 170 which defines the coding sequence portion of two exons from nucleic acids 1 through 169 (SEQ ID NO:29) and 170 through 588 (SEQ ID NO:30);

30 Figure 9 illustrates the mouse cDNA sequence containing a 5' non-coding region (SEQ ID NO:13) and a 3' non-coding region (SEQ ID NO:14) each of which are contiguous to the coding region of pre-pro neurturin;

Figure 10 illustrates the percent neuronal 35 survival in E18 rat nodose ganglia neurons treated 24 hours post-plating for NTN, GDNF, BDNF, NGF and AMO;

Figure 11 illustrates the nucleotide and amino acid sequence of murine persephin (SEQ ID NOS:79, 80 and 81; amino acid residues 52 through 140, 47 through 142, and 9 through 142, respectively);

5           Figure 12 illustrates the family member sequence identity in the region between the first and seventh canonical framework cysteine residues aligned beginning with the first canonical framework cysteine for murine GDNF (SEQ ID NO:87), murine neurturin (NTN) (SEQ ID  
10 NO:88) and murine persephin (PSP) (SEQ ID NO:89);

Figure 13 illustrates the partial sequence of rat persephin cDNA (SEQ ID NO:97) obtained by the technique of rapid amplification of cDNA ends;

          Figure 14 illustrates the partial sequence  
15 beginning with the first canonical framework cysteine for rat persephin (SEQ ID NO:83) and the corresponding polynucleotide sequence (SEQ ID NO:86);

          Figure 15 shows the family member aligned partial amino acid sequences from the first through the seventh  
20 canonical framework cysteine residues illustrating family member sequence homology of the mature growth factors, human GDNF, rat GDNF, mouse GDNF, human neurturin (NTN), mouse neurturin, rat persephin (PSP), and mouse persephin in which boxes enclose the 28 conserved amino acid  
25 residues present in all;

          Figure 16 illustrates the sequences of TGF- $\beta$  superfamily members aligned using the Clustal method, from the first canonical framework cysteine to the end of the sequence for transforming growth factor- $\beta$ 1 (TGF $\beta$ 1),  
30 transforming growth factor- $\beta$ 2 (TGF $\beta$ 2), transforming growth factor- $\beta$ 3 (TGF $\beta$ 3), inhibin  $\beta$  A (INH $\beta$ A), inhibin  $\beta$  B (INH $\beta$ B), the nodal gene (NODAL), bone morphogenetic proteins 2 and 4 (BMP2 and BMP4), the *Drosophila* decapentaplegic gene (*dpp*), bone morphogenetic proteins  
35 5-8 (BMP5, BMP6, BMP7 and BMP8), the *Drosophila* 60A gene family (60A), bone morphogenetic protein 3 (BMP3), the

Vg1 gene, growth differentiation factors 1 and 3 (GDF1 and GDF3), dorsalin (drsln), inhibin  $\alpha$  (INH $\alpha$ ), the MIS gene (MIS), growth factor 9 (GDF-9), glial-derived neurotrophic growth factor (GDNF) and neurturin (NTN);

5        Figure 17 illustrates full length murine persephin gene (SEQ ID NO:131), the amino acid sequence which includes at least a portion of the pre-pro region encoded by the nucleotide sequence in the first reading frame from the initiator methionine codon through the stop  
10 codon at nucleotide positions 244-246 (SEQ ID NO:132) and the amino acid sequence which includes mature persephin in the second reading frame from nucleotide position 2 through the stop codon at positions 557-559 (SEQ ID NO:133);

15        Figure 18 illustrates full length rat persephin gene (SEQ ID NO:134), the amino acid sequence which includes at least a portion of the pre-pro region encoded by the nucleotide sequence in the first reading frame from the initiator methionine codon through the stop  
20 codon at nucleotide positions 244-246 (SEQ ID NO:135) and the amino acid sequence which includes mature persephin in the second reading frame from nucleotide position 2 through the stop codon at positions 557-559 (SEQ ID NO:136);

25        Figure 19 illustrates a western blot analysis using anti-persephin antibodies to detect persephin protein in cell lysates from COS monkey cells transfected with the murine persephin gene (lane 2) or the rat persephin gene (lane 3) compared to cells transfected  
30 with the non-recombinant vector alone (pCB6, lane 4) and the mature protein produced by *E. Coli* (lane 1);

      Figure 20 illustrates the murine chimeric molecules (A) PSP/NTN containing the persephin fragment (residues 1-63) and the neurturin fragment (residues 68-  
35 100) and (B) NTN/PSP containing the neurturin fragment (residues 1-67) and the persephin fragment (residues 64-



96) with the arrow indicating the crossover point in each;

Figure 21 illustrates the survival promoting effect of persephin in murine embryonic day-14

5 mesencephalic cells cultured for three days (a) in the absence of persephin where almost all of the cells are dead and (b) in the presence of persephin (100 ng/ml) where substantial neuronal cell survival is evident; and

Figure 22 illustrates RT/PCT survey for persephin  
10 expression in adult mouse tissues showing persephin expression by Kidney cells.

#### Description of the Preferred Embodiments

The present invention is based upon the  
15 identification, isolation and sequencing of a DNA molecule that encodes a new growth factor, persephin. Because of the sequence similarity to neurturin and GDNF, persephin is believed to be capable of promoting cell survival and, in particular, the survival of neurons.  
20 Prior to this invention, persephin was unknown and had not been identified as a discrete biological substance nor had it been isolated in pure form.

The growth factor, neurturin (NTN) was identified and isolated as set forth in copending application Serial  
25 Number 08/519,777 filed August 28, 1995, which is incorporated in its entirety by reference. From the sequence of neurturin and the sequence of the closely related growth factor, glial-derived neurotrophic factor (GDNF), the inventors herein have devised and pursued  
30 strategies to find additional related factors. Neurturin is approximately 40% identical to GDNF, but less than 20% identical to any other member of the TGF- $\beta$  superfamily. Together these two proteins define a new subfamily within the TGF- $\beta$  superfamily. Several sequence regions within  
35 neurturin and GDNF were identified that are highly conserved, such that they are likely to be present in any

additional members of this subfamily. This sequence information can therefore be used to isolate previously unknown members of this subfamily by designing degenerate oligonucleotides to be used as either primers in PCR reactions or as probes in hybridization studies.

Using the new degenerate primer PCR strategy described in Example 11 of copending application Serial Number 08/519,777, the inventors herein have succeeded in identifying a third factor, persephin, that is approximately 40-50% identical to both GDNF and neurturin. Primers corresponding to the amino acid sequence from conserved regions of neurturin and GDNF (SEQ ID NO:42 and SEQ ID NO:44) were used to amplify a 77 nt fragment from rat genomic DNA. The resulting products were subcloned into the Bluescript KS plasmid and sequenced. The sequence of one of the amplified products predicted amino acid sequence data internal to the PCR primers that was different from that of GDNF or neurturin but had more than 20% identity with GDNF and neurturin, whereas the sequences of other amplified products we obtained corresponded to GDNF or neurturin, as would be expected. The 22 nucleotide sequence (SEQ ID NO:90) was then aligned with the rat sequences of GDNF and neurturin and found to be unique. This novel sequence, thus, suggested that we had identified a new family member referenced herein as persephin.

To obtain additional persephin sequence information, primers containing the unique 22 nucleotide sequence of the amplified fragment were used in the rapid amplification of cDNA ends (RACE) technique (Frohman, M.A. Methods in Enzymology 218:340-356, 1993) using cDNA obtained from neonatal rat brain. An approximately 350 nt fragment was obtained from this PCR reaction which constituted a partial rat persephin cDNA sequence of approximately 350 nucleotides (SEQ ID NO:106). The predicted amino acid sequence of this cDNA was compared

to that of GDNF and neurturin, and found to have approximately 40% identity with each of these proteins. Importantly, the characteristic spacing of the canonical framework cysteine residues in members of the TGF- $\beta$  superfamily was present. Furthermore, in addition to the region of similarity encoded by the degenerate primers used to isolate persephin, another region of high homology shared between GDNF and neurturin, but absent in other members of the TGF- $\beta$  superfamily, was also present in persephin

GDNF	ACCRPVAFDDDLFLDD	(aa 60-76) (SEQ ID NO:98)
NTN	PCCRPTAYEDEVSKDV	(aa 61-77) (SEQ ID NO:99)
PSP	PCCQPTSYAD-VTFLDD	(aa 57-72) (SEQ ID NO:100)

(Amino acid numbering uses the first Cys residue as amino acid 1).

With the confirmation that persephin was indeed a new member of the GDNF/NTN subfamily, we isolated murine genomic clones of persephin to obtain additional sequence information. Primers corresponding to rat cDNA sequence were used in a PCR reaction to amplify a 155 nucleotide (nt) fragment from mouse genomic DNA which was homologous to the rat persephin cDNA sequence. These primers were then used to obtain murine persephin genomic clones from a mouse 129/Sv library in a P1 bacteriophage vector (library screening service of Genome Systems, Inc., St. Louis, MO).

Restriction fragments (3.4 kb Nco I and a 3.3 kb Bam H1) from this P1 clone containing the persephin gene were identified by hybridization with a 210 nt fragment of persephin obtained by PCR using mouse genomic DNA and persephin-specific primers. The Nco I and Bam H1 fragments were sequenced and found to encode a stretch of amino acids corresponding to that present in the rat persephin RACE product, as well as being homologous to the mature regions of both neurturin and GDNF (Figure 11).

When the amino acid sequences of mature murine GDNF,

NTN and PSP are aligned using the first canonical framework cysteine as the starting point, which is done because alterations in the cleavage sites between family members creates variability in the segments upstream of the first cysteine, persephin (91 amino acids) is somewhat smaller than either neurturin (95 amino acids) or GDNF (94 amino acids). The overall identity within this region is about 50% with neurturin and about 40% with GDNF (Figure 12).

Further nucleotide sequencing of the murine persephin NcoI fragment revealed the nucleotide sequence of the entire murine persephin gene as shown in Figure 17. In addition, the entire rat persephin gene has been determined by sequencing a PCR amplified fragment of rat genomic DNA as shown in Figure 18. In both the murine and rat persephin gene, an open reading frame extends from the sequence coding for an initiator methionine up to a stop codon at positions 244-246. However, somewhere in this sequence an apparent anomaly occurs such that the sequence encoding the RXXR cleavage site (positions 257-268) and the sequence corresponding to the mature persephin protein (positions 269-556) are not co-linear with this open reading frame. Instead, a second reading frame encodes the cleavage site and the mature persephin. The two cogent reading frames are shown in Figures 17 and 18. Irrespective of this apparent anomaly, mammalian cells express persephin from either the murine or rat full length genomic sequence (see Example 14 below).

The N-terminus of persephin was predicted by reference to the N-terminal regions of neurturin or GDNF. Using neurturin sequence homology and cleavage signals, a characteristic RXXR cleavage motif is present beginning 9 residues upstream of the first canonical framework cysteine of persephin which would suggest that mature murine persephin would contain 5 amino acids (ALAGS) (SEQ ID NO:103) upstream of this cysteine (as does neurturin).

The corresponding 5 amino acids in rat persephin are ALPGL (SEQ ID NO:112). Using these parameters, mature persephin would consist of 96 amino acids and have a predicted molecular mass of 10.4 kD. Using GDNF sequence  
5 homology and cleavage signals, on the other hand suggests that the N-terminus upstream from the first cysteine of persephin could be longer, in accord with that observed for GDNF which is 40 residues. A characteristic RXXXR cleavage motif is thus located 47 residues upstream of  
10 the first cysteine and this would suggest that mature persephin would contain 43 amino acids (VRIPGGLPTPQFLLSKPSLCLTILLYLALGNNHVRLPRALAGS) (SEQ ID NO:104) upstream of this cysteine. Using these parameters, mature persephin would consist of 134 amino  
15 acids and have a predicted molecular mass of 14.5 kD. Thus, mature persephin may exist in either or both of the 96 amino acid predicted 10.4 kD or for the 134 amino acid predicted 14.5 kD form.

By "mature" growth factor reference is made to the  
20 secreted form of the growth factor in which any pre- or pro- regions have been cleaved and which may exist as a monomer or, by analogy to other members of the TGF- $\beta$  superfamily, in the form of a homodimer linked by disulfide bonds.

25 The discovery of the new growth factor, persephin, as described above is a result of the prior discovery by the inventors herein of neurturin. Thus, the experiments leading to the discovery of neurturin are relevant to the current discovery of persephin and to the predicted human  
30 form of persephin as well as to the biological activity of persephin.

Neurturin was identified and isolated by the inventors herein from conditioned medium for CHO cells. The initial neuronal survival promoting activity was  
35 identified by the inventors in a partially purified preparation of this CHO-conditioned medium. Preparation

of conditioned medium for a given cell line is well known in the art (for example, see Reid, in *Methods in Enzymology Vol. LVIII, Cell Culture*, Jakoby and Pastan, Eds., Academic Press, San Diego, pp 161-164, 1979;

5 Freshney, *Culture of Animal Cells in A Manual of Basic Technique, 2d Ed.*, Wiley-Liss, NY, p. 84, 1987 which are incorporated by reference). Thus, although in the present work CHO cells were cultured and the conditioned medium used to identify and to obtain neurturin in

10 purified form, one skilled in the art will readily appreciate that any cell that expresses neurturin can be used as a source. Some of the cells that express neurturin are identified below in Example 9 and the inventors herein believe that any of the cells identified

15 as expressing neurturin can be used to obtain conditioned medium from which neurturin can be isolated.

In the isolation of neurturin from the CHO cell conditioned medium, an initial crude conditioned medium can be obtained by centrifugation and/or filtration to

20 remove cellular debris. For further purification, one skilled in the art will readily appreciate that any of a number of methods known in the art can be used to isolate and purify neurturin from a biological sample such as affinity chromatography, ion exchange chromatography,

25 preparative electrophoresis or the like wherein the methods are used either individually or in combination.

The cell survival promoting effect of neurturin can be assessed in any suitable system for assessing cell survival. The inventors herein believe that neurturin

30 can promote survival in a variety of different tissues based upon what is known for other growth factors and upon the observation that neurturin is expressed in a number of tissues in which it is believed to have a survival promoting effect.

35 By virtue of the degree of sequence identity of persephin with its paralogs, neurturin and GDNF and the

known actions of these substances in promoting the survival and growth of neuronal and non-neuronal tissues, it is also believed that persephin will promote survival and growth in neuronal tissues as well as a variety of  
5 non-neuronal tissues. Indeed, the inventors herein have identified brain, kidney, and heart tissues as tissues expressing persephin which further supports the conclusion that persephin can act to promote growth and survival in neuronal and non-neuronal cells.

10 In the work reported herein, neuronal activity for neurturin was assessed using a sympathetic neuronal survival assay (sympathetic cervical ganglia, SCG) which has been extensively characterized (Martin et al, *J Cell Biol* 106:829-844, 1989; Deckwerth and Johnson, *J Cell*  
15 *Biol* 123:1207-1222, 1993 which are incorporated by reference) (see Figure 3). We also show the survival promoting effects of neurturin on sensory neurons (See Figure 10). In the same sympathetic and sensory neuronal cell assays, persephin showed little survival  
20 potentiating activity. However, in a preparation of CNS neuronal cells of mesencephalic origin, persephin showed neuronal survival potentiating activity. This suggests that persephin will be applicable in the treatment or prevention of diseases involving neuronal degeneration in  
25 the CNS such as, for example, Parkinson's disease.

By way of illustrating the methods used in the above survival assays, the SCG assay involved the culturing of cells obtained from superior cervical ganglia of rat embryo for 5 days at 37°C in medium containing nerve  
30 growth factor (NGF). The medium was then exchanged with a medium containing no NGF and containing anti-NGF antiserum. Removal of NGF results normally in death of the neurons in 24-72 hours. Neuronal survival was visually assessed under a microscope on days 7-8.  
35 Maximum neuronal survival criteria included lack of degeneration of both neuronal cell bodies and neurites.

Cell body degeneration was indicated when the neuronal cell body was reduced in size, showed irregular membrane swellings, contained vacuoles, or had lost refractility. A field of neurites was scored as showing signs of  
5 disintegration when swellings and blebs appeared along the neurite bundles. Survival was determined by comparison with neurons grown in the presence of NGF (positive control) or in the absence of NGF with NGF antisera (negative control).

10 Activity was quantitated by calculation of a "survival unit". The total survival units in a sample were defined as the minimal volume of an aliquot of the sample which produced maximal survival divided into the total volume of that sample. For example, a volume of  
15 600 ml was eluted from the heparin agarose column and from this eluate, 12.5  $\mu$ l was the minimum volume that promoted maximal volume. Thus, the survival units in the eluate from the heparin agarose column was 48,000. Specific activity was calculated as the survival units  
20 divided by the mg total protein. The intrinsic activity of neurturin is expressed herein in concentration units of pg/ml or pM promoting maximal or half-maximal survival. As shown in Figure 5, a concentration-response curve of purified neurturin protein indicates that the  
25 intrinsic activity of neurturin expressed as an  $EC_{50}$  is approximately 1.5 ng/ml or approximately 50 pM and an  $EC_{100}$  is approximately 3 ng/ml or approximately 100 pM.

Survival units were determined in an assay using approximately 1200 neurons in a 0.5 ml culture assay and  
30 a culture period of 48 hours following addition of the fraction. Survival was assessed visually after the 48 hours. Intrinsic activity as shown in Figure 4 was determined in an assay using approximately 2700 neurons and a culture period of 72 hours. Survival was assessed  
35 by fixing the neurons and counting the number of surviving neurons. Because the stability, as assessed by



half-life of activity, for neurturin decreases as the number of neurons increases, the intrinsic activity measurement would be expected to be lower than that predicted by Specific Activity determinations. The

5 intrinsic activity measurement would also be expected to be lower than that predicted by specific activity because the survival was measured after 72 hours instead of 48 hours.

The purification of neurturin is described in detail  
10 in Example 1 below. The conditioned medium starting material was prepared from a derivative of DG44 Chinese hamster ovary cells, DG44CHO-pHSP-NGFI-B (Day et al, *J Biol Chem* 265:15253-15260, 1990 which is incorporated by reference). The inventors herein have also isolated  
15 neurturin in partially purified form from conditioned medium of other derivatives of DG44 Chinese hamster ovary cells and these other cells could be used equally as well as the DG44CHO-pHSP-NGFI-B cells as could the parent DG44 Chinese hamster ovary Cells, ovary cells from other  
20 species and cells from other tissues such as those known to express neurturin (See example 9). In preparing the conditioned medium, cells were placed in serum free medium for 2 days at which time conditioned medium is collected and the medium replenished. This cycle was  
25 repeated to yield 5 harvests of conditioned medium from each batch of CHO cells. The collected media was centrifuged to remove cellular debris.

The first step in purification of neurturin from the CHO cell conditioned medium involved the introduction of  
30 the conditioned medium onto a heparin agarose column and the elution of partially purified neurturin therefrom. This step resulted in an 111 fold increase in the specific activity and purification of the protein. The buffer used to apply the medium to the column contains  
35 0.5 M NaCl. At this concentration of NaCl the neurturin binds to the heparin agarose matrix. The inventors

herein believe that based upon their isoelectric points, LIF and CNTF would either not bind to the heparin agarose matrix or be washed away from the matrix with buffer containing 0.5 M NaCl. Thus, this step would be expected  
5 to isolate neurturin from growth factors such as LIF and CNTF. After washing the column, neurturin was eluted from the column using 1.0 M NaCl.

For further purification, the eluted material was then diluted and introduced into a column containing SP  
10 SEPHAROSE® High Performance ion exchange resin (Pharmacia, Piscataway, NJ). Material eluted from this column was further purified using fast protein liquid chromatography (FPLC) on a Chelating Superose HR 10/2 column charged with Cu<sup>++</sup> (Pharmacia, Piscataway, NJ).  
15 Eluted fractions from the Cu<sup>++</sup> superose column were introduced into a Mono S HR 5/5 cation exchange column (Pharmacia, Piscataway, NJ) for further FPLC purification. The composition of the proteins in the Mono S fractions were analyzed using non-reducing SDS-  
20 PAGE and silver staining.

Fractions collected from the columns at each stage of purification were assayed for biological activity using the neuronal survival assay and for protein content using the dye binding method of Bradford (*Anal Biochem*  
25 72:248-254, 1976 which is incorporated by reference) with a Bio-Rad protein assay dye reagent (Bio-Rad Laboratories, Inc., Hercules, CA). The progressive purification using the above steps is shown in table 1.

Table 1

		Protein <sup>a</sup> (mg)	Activity <sup>b</sup> (units)	Specific Activity <sup>d</sup> (units/mg)	Yield (%)	Purification (fold)
5	Conditioned Medium	5000	48000 <sup>c</sup>	9.6	-	-
	Heparin Agarose	45	48000	1068	100	111
	SP Sepharose	5.3	48000	9058	100	943
10	Cu++ Superose	0.31	30000	96700	62	10070
	Mono S	0.004	15000	3750000	31	390000

a. mg protein was determined using the dye binding method of Bradford (*Anal Biochem* 72:248, 1976).

b. The total activity units or survival units in a sample were defined as the minimal volume of an aliquot of the sample which produced maximal survival divided into the total volume of that sample.

c. Activity for Conditioned Medium was derived from the assumption that 100% of the activity was recovered in the heparin agarose fraction because the activity of conditioned medium was too low to be directly assayed.

d. Specific Activity was the Activity units divided by the mg total protein.

The results of this analysis along with the results of the neuronal survival assay of fractions revealed that a protein having an apparent molecular weight of about 25 kD co-purified with the sympathetic neuron survival activity.

The purified material isolated from CHO cell conditioned medium was used to determine partial amino acid sequences of the protein in CHO cell conditioned medium and subsequently as a basis for determining the sequences in different species. The N-terminal amino acid sequence was determined using an automated protein/peptide sequencer and the first 16 amino acids

were considered to be, with uncertainty as to position 6, Ser-Gly-Ala-Arg-Pro-Xaa-Gly-Leu-Arg-Glu-Leu-Glu-Val-Ser-Val-Ser where Xaa was an unknown amino acid (SEQ ID NO:3). Internal amino acid fragments were obtained from the purified material following digestion with protease enzymes and the sequences determined. Three internal fragments thus obtained were (1) with uncertainty as to positions 1, 2 and 6, Xaa<sub>1</sub>-Cys-Ala-Gly-Ala-Xaa<sub>2</sub>-Glu-Ala-Ala-Val where Xaa<sub>1</sub> was unknown amino acid, Xaa<sub>2</sub> was Ser or Cys (SEQ ID NO:4); (2) with uncertainty as to positions 1, 2, 4, 10, 17 and 22, Xaa<sub>1</sub>-Xaa<sub>2</sub>-Val-Glu-Ala-Lys-Pro-Cys-Cys-Gly-Pro-Thr-Ala-Tyr-Glu-Asp-Xaa<sub>3</sub>-Val-Ser-Phe-Leu-Ser-Val where Xaa<sub>1</sub> and Xaa<sub>2</sub> were unknown, Xaa<sub>3</sub> was Gln or Glu (SEQ ID NO:5) and (3) Tyr-His-Thr-Leu-Gln-Glu-Leu-Ser-Ala-Arg (SEQ ID NO:6). Based upon these partial amino acid sequences, DNA probes and primers can be made and used to obtain cDNA clones from different species based upon high sequence conservation between mammalian species. The human cDNA and inferred amino acid sequence is shown in Figure 7 and the mouse cDNA and inferred amino acid sequence is shown in Figure 8.

The cDNA clone from mouse was 1.0 kb having an open reading frame of 585 nucleotides (SEQ ID NO:12) encoding the mouse pre-pro neurturin protein (SEQ ID NO:8, Figure 8). In addition, non-coding regions have been identified at both the 5' and 3' ends of the coding region as shown in Figure 9. (SEQ ID NO:13, 5' non-coding region, nucleic acids -348 through -1; SEQ ID NO:14, 3' non-coding region, nucleic acids 589 through 675). The mouse neurturin sequence can be used to obtain PCR primers for use in identifying homologs from other species. A human 192 nucleotide fragment from human genomic DNA was amplified by this method and further used to screen a human genomic library to obtain clones containing the human neurturin genomic locus. The human cDNA sequence was deduced from the sequencing of these

clones. (Figure 7, cDNA sequence of human pre-pro neurturin).

Reference to persephin or to neurturin herein is intended to be construed to include growth factors of any  
5 origin which are substantially homologous to and which are biologically equivalent, respectively, to the persephin characterized and described herein or to the neurturin characterized and described herein. Such substantially homologous growth factors may be native to  
10 any tissue or species and, similarly, biological activity can be characterized in any of a number of biological assay systems. Reference to pre-pro neurturin herein is intended to be construed to include pre-pro growth factors containing a pre- or leader or signal sequence  
15 region, a pro- sequence region and neurturin as defined herein.

The terms "biologically equivalent" are intended to mean that the compositions of the present invention are capable of demonstrating some or all of the same  
20 growth properties in a similar fashion, not necessarily to the same degree as the neurturin isolated from the CHO cell conditioned medium herein or recombinantly produced human or mouse or rat neurturin or persephin as the case may be.

25 By "substantially homologous" it is meant that the degree of sequence identity of neurturin orthologs including human and mouse neurturin as well as neurturin from any other species or the degree of sequence identity of persephin orthologs including human, mouse and rat  
30 persephin as well as persephin from any other species, is greater than that between paralogs such as persephin and neurturin or persephin and GDNF, and greater than that reported previously for members of the TGF- $\beta$  superfamily (For discussion of homology of TGF- $\beta$  superfamily members  
35 see Kingsley, Genes and Dev 8:133-46, 1994 which is incorporated by reference).

Sequence identity or percent identity is intended to mean the percentage of same residues between two sequences. The reference sequence is mouse persephin when determining percent identity with mouse GDNF and mouse neurturin and rat persephin when determining percent identity with rat GDNF and rat neurturin. Referencing is to human neurturin when determining percent identity with non-human neurturin, to human neurturin when determining percent identity with non-neurturin growth factors and to human GDNF when determining percent identity of non-neurturin growth factors with GDNF. In all of the above comparisons, the two sequences being compared are aligned using the Clustal method (Higgins et al, *Cabios* 8:189-191, 1992) of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, WI). In this method, multiple alignments are carried out in a progressive manner, in which larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues in the alignment, determined from a residue weight table representing the probability of a given amino acid change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment = 10; gap length penalty for multiple alignment = 10; k-tuple value in pairwise alignment = 1; gap penalty in pairwise alignment = 3; window value in pairwise alignment = 5; diagonals saved in pairwise alignment = 5. The residue weight table used for the alignment program is PAM250 (Dayhoff et al., in *Atlas of*

*Protein Sequence and Structure*, Dayhoff, Ed., NBRF, Washington, Vol. 5, suppl. 3, p. 345, 1978).

Percent conservation is calculated from the above alignment by adding the percentage of identical residues to the percentage of positions at which the two residues represent a conservative substitution (defined as having a log odds value of greater than or equal to 0.3 in the PAM250 residue weight table). Conservation is referenced to mouse persephin when determining percent conservation with persephin from other species or with non-persephin growth factors; referenced to human neurturin when determining percent conservation with non-human neurturin or with non-neurturin growth factors, and referenced to human GDNF when determining percent conservation to non-persephin, non-neurturin growth factors with GDNF. Conservative amino acid changes satisfying this requirement are: R-K; E-D, Y-F, L-M; V-I, Q-H.

Table 2 shows the calculations of identity (I) and conservation (C) for comparisons of persephin and neurturin and GDNF from various species. Comparisons were made between mouse persephin from the first canonical framework cysteine to the end (SEQ ID NO:89) and rat persephin from the first canonical framework cysteine to the end (SEQ ID NO:83); between mouse persephin and mouse GDNF from the first cysteine to the end (mGDNF/C-END, SEQ ID NO:87) or mouse neurturin from the first cysteine to the end (mNTN/C-END, SEQ ID NO:88); and between rat persephin and rat GDNF from the first cysteine to the end (rGDNF/C-END). Neurturin comparisons were between mature human and mature mouse neurturin (hNTN and mNTN, respectively) and between each of these and mature human, rat and mouse GDNF (hGDNF, rGDNF and mGDNF, respectively) as shown in the table.

Table 2.

	COMPARISON	% IDENTITY	% CONSERVATION
5	mPSP v. rPSP	96	98
	mPSP v. mNTN/C-END	51	54
	mPSP v. mGDNF/C-END	41	46
	rPSP v. rGDNF/C-END	42	45
10	hNTN v. mNTN	90	93
	hNTN v. rGDNF	44	53
	hNTN v. mGDNF	43	52
	hNTN v. hGDNF	43	53
	mNTN v. rGDNF	42	52
15	mNTN v. mGDNF	41	51
	mNTN v. hGDNF	41	52

The degree of homology between the mouse persephin  
 and rat persephin is about 96% and it is believed that  
 the degree of homology between either mouse or rat  
 persephin and human persephin is at least about 85%  
 identity based upon a similar comparison with neurturin.  
 The neurturin comparisons as shown in Table 2 indicate  
 mature mouse and human neurturin proteins have about 90%  
 sequence identity. Furthermore, all persephin and  
 neurturin homologs of non-human mammalian species are  
 believed to similarly have at least about 85% sequence  
 identity with human persephin and neurturin,  
 respectively. For non-mammalian species such as avian  
 species, it is believed that the degree of homology with  
 persephin or neurturin is at least about 65% identity  
 with human persephin or human neurturin, respectively.  
 By way of comparison, the variations between family  
 members of the neurturin-persephin-GDNF family of growth  
 factors can be seen by the comparison of persephin and  
 GDNF or neurturin and GDNF. Mouse and rat persephin have  
 about 35 to 40% sequence identity with mouse and rat GDNF  
 respectively. Similarly, human and mouse neurturin have  
 about 40% sequence identity and about 50% sequence  
 conservation with human, mouse and rat GDNF. It is  
 believed that the different family members also have a



similar sequence identity of about 40% of that of neurturin, about 40% of that of persephin and about 40% of that of GDNF and within a range of about 30% to about 85% identity with neurturin, within a range of about 30% to about 85% identity with persephin and within a range of about 30% to about 85% sequence identity with GDNF. Thus, a given member of the GDNF-neurturin-persephin family would be expected to have lesser sequence identity with any other family member of the same species than is present in orthologs of that family member in other species just as human GDNF and human neurturin are more closely related to mouse GDNF and mouse neurturin, respectively, than to each other or to GDNF and any given family member would be expected to have greater sequence identity with another family member than to any other known member of the TGF- $\beta$  superfamily (Kingsley, *supra*).

In the case of pre-pro neurturin, homologs of pre-pro neurturin in non-human mammalian species can be identified by virtue of the neurturin portion of the amino acid sequence having at least about 85% sequence identity with human neurturin and homologs of pre-pro neurturin in non-mammalian species can be identified by virtue of the neurturin portion of the amino acid sequence having at least about 65% identity with human neurturin. It is similarly believed that mammalian pre-pro persephin proteins including the human ortholog will have at least about 85% sequence identity in the mature persephin portion of the molecule and that non-mammalian pre-pro persephin proteins will have at least about 65% sequence identity with human pre-pro persephin.

Either persephin or neurturin, as the terms are used herein, can also include hybrid and modified forms of persephin or neurturin, respectively, including fusion proteins and persephin or neurturin fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced and modifications such as where one

or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosolations so long as the hybrid or modified form retains the biological activity of persephin or

5 neurturin. By retaining the biological activity, it is meant that neuronal survival is promoted, although not necessarily at the same level of potency as that of the neurturin isolated from CHO cell conditioned medium or that of the recombinantly produced human or mouse

10 neurturin or human or mouse or rat persephin.

Also included within the meaning of substantially homologous is any persephin or neurturin which may be isolated by virtue of cross-reactivity with antibodies to the persephin or neurturin, respectively, as described

15 herein or whose encoding nucleotide sequences including genomic DNA, mRNA or cDNA may be isolated through hybridization with the complementary sequence of genomic or subgenomic nucleotide sequences or cDNA of the persephin or neurturin, respectively, as described herein

20 or fragments thereof. It will also be appreciated by one skilled in the art that degenerate DNA sequences can encode human neurturin or human persephin and these are also intended to be included within the present invention as are allelic variants of neurturin or persephin,

25 respectively.

In the case of pre-pro neurturin, alternatively spliced protein products resulting from an intron located in the coding sequence of the pro region may exist. The intron is believed to exist in the genomic sequence at a

30 position corresponding to that between nucleic acids 169 and 170 of the cDNA which, in turn, corresponds to a position within amino acid 57 in both the mouse and human pre-pro neurturin sequences (see Figures 7 and 8). Thus, alternative splicing at this position might produce a

35 sequence that differs from that identified herein for human and mouse pre-pro neurturin (SEQ ID NO:11 and SEQ

ID NO:12, respectively) at the identified amino acid site by addition and/or deletion of one or more amino acids. Any and all alternatively spliced pre-pro neurturin proteins are intended to be included within the terms  
5 pre-pro neurturin as used herein.

Although it is not intended that the inventors herein be bound by any theory, it is thought that the human and mouse proteins identified herein as well as homologs from other tissues and species may exist as  
10 dimers in their biologically active form in a manner consistent with what is known for other factors of the TGF- $\beta$  superfamily.

In addition to homodimers, the monomeric units of the dimers of neurturin or persephin can be used to  
15 construct stable growth factor heterodimers or heteromultimers comprising at least one monomer unit derived from persephin or at least one monomer unit derived from neurturin. This can be done by dissociating a homodimer of neurturin or a homodimer of persephin into  
20 its component monomeric units and reassociating in the presence of a monomeric unit of a second or subsequent homodimeric growth factor. This second or subsequent homodimeric growth factor can be selected from a variety of growth factors including neurturin, persephin, GDNF, a  
25 member of the NGF family such as NGF, BDNF, NT-3 and NT-4/5, a member of the TGF- $\beta$  superfamily, a vascular endothelial growth factor, a member of the CNTF/LIF family and the like.

Growth factors are thought to act at specific  
30 receptors. For example, the receptors for TGF- $\beta$  and activins have been identified and make up a family of Ser/Thr kinase transmembrane proteins (Kingsley, *Genes and Dev* 8:133-146, 1994; Bexk et al *Nature* 373:339-341, 1995 which are incorporated by reference). In the NGF  
35 family, NGF binds to the TrkA receptor in peripheral sensory and sympathetic neurons and in basal forebrain

neurons; BDNF and NT-4/5 bind to trkB receptors; and NT-3 binds primarily to trkC receptors that possess a distinct distribution within the CNS (Tuszynski et al., *Ann Neurol* 35:S9-S12, 1994). The inventors herein believe that

5 persephin, neurturin, GDNF and as yet unknown members of this family of growth factors act through specific receptors having distinct distributions as has been shown for other growth factor families. These may be separate receptors or it is also possible that members of the

10 GDNF-neurturin-persephin family may act upon the same receptor as is the case with BDNF and NT-4/5 which act on the trkB receptor. Nevertheless, by forming heterodimers or heteromultimers of persephin or neurturin and one or more other growth factors, the resultant growth factor

15 would be expected to be able to bind to at least two distinct receptor types preferentially having a different tissue distribution. The resultant heterodimers or heteromultimers would be expected to show an enlarged spectrum of cells upon which it could act or provide

20 greater potency. It is also possible that the heterodimer or heteromultimer might provide synergistic effects not seen with homodimers or homomultimers. For example, the combination of factors from different classes has been shown to promote long-term survival of

25 oligodendrocytes whereas single factors or combinations of factors within the same class promoted short-term survival (Barres et al., *Development* 118:283-295, 1993).

Heterodimers can be formed by a number of methods. For example, homodimers can be mixed and subjected to

30 conditions in which dissociation/unfolding occurs, such as in the presence of a dissociation/unfolding agent, followed by subjection to conditions which allow monomer reassociation and formation of heterodimers. Dissociation/unfolding agents include any agent known to

35 promote the dissociation of proteins. Such agents include, but are not limited to, guanidine hydrochloride,

urea, potassium thiocyanate, pH lowering agents such as buffered HCl solutions, and polar, water miscible organic solvents such as acetonitrile or alcohols such as propanol or isopropanol. In addition, for homodimers  
5 linked covalently by disulfide bonds as is the case with TGF- $\beta$  family members, reducing agents such as dithiothreitol and  $\beta$ -mercaptoethanol can be used for dissociation/unfolding and for reassociation/refolding.

Heterodimers can also be made by transfecting a cell  
10 with two or more factors such that the transformed cell produces heterodimers as has been done with the neurotrophins. (Heymach and Shooter, *J Biol Chem* 270:12297-12304, 1995).

Another method of forming heterodimers is by  
15 combining persephin or neurturin homodimers and a homodimer from a second growth factor and incubating the mixture at 37°C.

When heterodimers are produced from homodimers, the heterodimers may then be separated from homodimers using  
20 methods available to those skilled in the art such as, for example, by elution from preparative, non-denaturing polyacrylamide gels. Alternatively, heterodimers may be purified using high pressure cation exchange chromatography such as with a Mono S cation exchange  
25 column or by sequential immunoaffinity columns.

It is well known in the art that many proteins are synthesized within a cell with a signal sequence at the N-terminus of the mature protein sequence and the protein carrying such a leader sequence is referred to as a  
30 preprotein. The pre- portion of the protein is cleaved during cellular processing of the protein. In addition to a pre- leader sequence, many proteins contain a distinct pro sequence that describes a region on a protein that is a stable precursor of the mature protein.  
35 Proteins synthesized with both pre- and pro- regions are referred to as preproteins. In view of the processing

events known to occur with other TGF- $\beta$  family members as well as the sequences determined herein, the inventors believe that the forms of persephin or neurturin protein as synthesized within a cell is the pre-pro persephin or a pre-pro neurturin. In the case of neurturin, the pre-pro neurturin is believed to contain an N-terminal 19 amino acid signal sequence (human pre- signal sequence, SEQ ID NO:15, Figure 7, amino acids 1 through 19 encoded by SEQ ID NO:17, Figure 7, nucleic acids 1 through 57; mouse pre- signal sequence, SEQ ID NO:16, Figure 8, amino acids 1 through 19, encoded by SEQ ID NO:18, Figure 8, nucleic acids 1 through 57). It is known that the full length of a leader sequence is not necessarily required for the sequence to act as a signal sequence and, therefore, within the definition of pre- region of neurturin is included fragments thereof, usually N-terminal fragments, that retain the property of being able to act as a signal sequence, that is to facilitate co-translational insertion into the membranes of one or more cellular organelles such as endoplasmic reticulum, mitochondria, golgi, plasma membrane and the like.

The neurturin signal sequence is followed by a pro-domain which contains an RXXR proteolytic processing site immediately before the N-terminal amino acid sequence for the mature neurturin. (human pro- region sequence, SEQ ID NO:19, Figure 7, amino acids 20 through 95 encoded by the nucleic acid sequence SEQ ID NO:20, Figure 7 nucleic acids 58 through 285; mouse pro- region sequence, SEQ ID NO:22, Figure 8, amino acids 19 through 95 encoded by nucleic acid sequence SEQ ID NO:21, Figure 8, nucleic acids 58 through 285).

The neurturin pre- and pro- regions together comprise a pre-pro sequence identified as the human pre-pro sequence (SEQ ID NO:23, Figure 7, amino acids 1 through 95 encoded by SEQ ID NO:25, nucleic acids 1 through 285) and the mouse pre-pro sequence (SEQ ID

NO:24, Figure 8, amino acids 1 through 95 encoded by SEQ ID NO:26, nucleic acids 1 through 285). The pre- region sequences and pro- region sequences as well as the pre-pro region sequences can be identified and obtained for non-human mammalian species and for non-mammalian species by virtue of the sequences being contained within the pre-pro neurturin as defined herein. It is believed that persephin is similarly associated with pre- and pro-regions to constitute a pre-pro persephin sequence.

Using the above landmarks, the mature, secreted neurturin molecule is predicted to be approximately 11.5 kD which is likely to form a disulfide linked homodimer of approximately 23 kD by analogy to other members of the TGF- $\beta$  family. The predicted approximately 23 kD protein is consistent with the 25 kD neurturin protein purified from CHO cell conditioned media being a homodimer. The inventors herein have detected an approximately 11.5 kD neurturin protein from conditioned medium of Chinese hamster ovary cells transfected with the neurturin expression vector (pCMV-NTN-3-1) using SDS-PAGE under reducing conditions and this protein is thought to be the monomer.

As discussed above, a mature persephin molecule predicted on the basis of homology to neurturin would contain 5 amino acids upstream from the first canonical framework cysteine thus having 96 amino acids and a predicted molecular mass of 10.4 kD. A mature persephin molecule based upon homology to GDNF would contain 43 amino acids upstream from the first canonical framework cysteine thus having 134 amino acids and a predicted molecular mass of 14.5 kD.

The nucleotide sequences of neurturin pre- and/or pro- regions or similar regions that are believed to be associated with persephin DNA can be used to construct chimeric genes with the coding sequences of other growth factors or proteins and, similarly, chimeric genes can be

constructed from the coding sequence of neurturin coupled to sequences encoding pre- and/or pro- regions from genes for other growth factors or proteins. (Booth et al., *Gene* 146:303-8, 1994; Ibanez, *Gene* 146:303-8, 1994;

- 5 Storic et al., *FEBS Letters* 337:303-7, 1994; Sha et al *J Cell Biol* 114:827-839, 1991 which are incorporated by reference). Such chimeric proteins can exhibit altered production or expression of the active protein species.

A preferred neurturin has been identified and  
10 isolated in purified form from medium conditioned by CHO cells. Also preferred is neurturin prepared by recombinant DNA technology. Similarly, a preferred persephin according to the present invention is prepared by recombinant DNA technology.

- 15 By "pure form" or "purified form" or "substantially purified form" it is meant that a persephin or neurturin composition is substantially free of other proteins which are not persephin or neurturin, respectively.

Recombinant persephin or neurturin may be made by  
20 expressing the DNA sequences encoding persephin or neurturin, respectively, in a suitable transformed host cell. Using methods well known in the art, the DNA encoding persephin or neurturin may be linked to an expression vector, transformed into a host cell and  
25 conditions established that are suitable for expression of persephin or neurturin, respectively, by the transformed cell.

Any suitable expression vector may be employed to produce recombinant human persephin or recombinant human  
30 neurturin such as, for example, the mammalian expression vector pCB6 (Brewer, *Meth Cell Biol* 43:233-245, 1994) or the *E. coli* pET expression vectors, specifically, pET-30a (Studier et al., *Methods Enzymol* 185:60-89, 1990 which is incorporated by reference) both of which were used  
35 herein. Other suitable expression vectors for expression in mammalian and bacterial cells are known in the art as



are expression vectors for use in yeast or insect cells. Baculovirus expression systems can also be employed.

Persephin or neurturin may be expressed in the monomeric units or such monomeric form may be produced by  
5 preparation under reducing conditions. In such instances refolding and renaturation can be accomplished using one of the agents noted above that is known to promote dissociation/association of proteins. For example, the monomeric form can be incubated with dithiothreitol  
10 followed by incubation with oxidized glutathione disodium salt followed by incubation with a buffer containing a refolding agent such as urea.

In the case of neurturin, by analogy with the N-terminal sequence and internal fragments of the neurturin  
15 purified from CHO cell conditioned medium, the mature mouse sequence was deduced and from this the mature human form was predicted using the sequence from the human gene. The amino acid sequence of the mature human form is as shown in Figure 5 (hNTN, SEQ ID NO:1). The  
20 material purified from CHO cell conditioned medium is considered to be mature neurturin and may exist as a dimer or other multimer and may be glycosylated or chemically modified in other ways.

Persephin, like neurturin, may also exist as a dimer  
25 or other multimer and may be glycosylated or chemically modified in other ways.

As noted above, the mouse and human nucleic acid sequences suggest that neurturin is initially translated as a pre-pro polypeptide and that proteolytic processing  
30 of the signal sequence and the "pro" portion of this molecule results in the mature sequence, referenced herein as "mature neurturin", as obtained from medium condition by CHO cells and as exists in human and in non-human species in homologous form. Neurturin, therefore,  
35 includes any and all "mature neurturin" sequences from human and non-human species and any and all pre-pro

neurturin polypeptides that may be translated from the neurturin gene.

As with neurturin, the persephin in the present invention also includes any and all mature persephin  
5 sequences from human and non-human species and any and all pre-pro persephin polypeptides that may be translated from the persephin gene.

It is believed that the coding sequence for the pre-pro-neurturin polypeptide begins at the first ATG codon  
10 encoding methionine at the 5' end of the clone (position 1 in figure 9) which is positioned in the same reading frame as the sequence encoding the amino acid sequences obtained from the purified neurturin. Downstream from the first codon is the largest open reading frame  
15 containing the coding sequence for the pre- and pro-regions followed by the coding sequence for the mature mouse neurturin.

Sequence analysis of the murine neurturin genomic clones identified a 0.5 kb intron located between  
20 nucleotide 169 and 170 of the pre-pro neurturin from the cDNA clones. This intron is located in the coding sequence of the pro- region of the pre-pro-neurturin protein. Thus, it is believed that the mouse neurturin gene contains at least two exons, one of which contains  
25 the coding sequences upstream from the splice site and the other contains the coding sequence downstream (Figure 8, SEQ ID NO:29, SEQ ID NO:30). It is known that the gene for GDNF contains an intron located at an analogous position and an alternately spliced form of GDNF has been  
30 detected by RT-PCR experiments (Suter-Crazzolara and Unsicker, *Neuroreport* 5: 2486-2488, 1994 which is incorporated by reference). This alternate form results from the use of a splice site in the second coding exon located 78 bp 3' to the original splice site reported.  
35 The alternately spliced form encodes a GDNF protein with a deletion of 26 amino acids relative to the originally

reported form. The two forms are expressed in different ratios in different tissues. We have not detected alternately spliced forms of neurturin in RT-PCR and RACE experiments using mouse P1 brain and P1 liver cDNAs. The  
5 possibility exists, however, that alternate splice sites in the neurturin gene may be utilized in different tissues.

The coding sequence of the human neurturin cDNA has been deduced from the sequence of the human neurturin  
10 genomic clones. The coding sequence of the human cDNA, like that of the mouse cDNA, is interrupted by an intron between nucleotides 169 and 170 of the coding sequence. Thus, the human neurturin gene is believed to contain at least two exons, one of which contains the coding  
15 sequence upstream from the splice site and the other contains the coding sequence downstream (Figure 7, SEQ ID NO:27, SEQ ID NO:28). The splice sites at the intron-exon junctions of the human and mouse genes have been conserved.

20 From the deduced amino acid sequence of human neurturin, the earlier predicted N-terminal sequence lies between positions 286 and 339 and the predicted internal sequences lie between positions 385 and 417, positions 474 and 533, and positions 547 and 576. The TGA stop  
25 codon at positions 592-594 terminate the open reading frame.

The predicted length of the purified pre-pro neurturin is 197 amino acid residues for the human pre-pro neurturin (SEQ ID NO:7) and 195 amino acid residues  
30 for the mouse pre-pro neurturin (SEQ ID NO:8). The predicted molecular weight of this polypeptide is 22.2 kD for mouse and 22.4 kD for human. The predicted length of the purified neurturin is 100 amino acid residues and its predicted monomeric molecular weight is 11.5 kD. There  
35 are no N-linked glycosylation sites, however, potential O-linked glycosylation sites occur at amino acid residues

in positions 18, 26, 80, 86 and 95 in human neurturin. Glycosylation at any one or combination of these sites would increase the molecular weight of the molecule.

In the case of persephin, there are no N-linked glycosylation sites in the region between the first and seventh canonical framework cysteines (SEQ ID NO:79) nor are there any N-linked glycosylation sites in a mature persephin molecule predicted on the basis of homology to neurturin (SEQ ID NO:80). In a mature persephin molecule based upon homology to GDNF there are two potential N-linked glycosylation sites in the 43 amino acids upstream from the first canonical framework cysteine at positions 31 and 32 in SEQ ID NO:81 (corresponding to positions 39 and 40 in the sequence as shown in Figure 11).

Potential O-linked glycosylation sites occur in persephin in the region between the first and seventh canonical framework cysteines at positions 5, 7, 19, 31, 38, 41, 62, 63, 68 and 83 in SEQ ID NO:79 (Figure 12) and in a mature persephin molecule predicted on the basis of homology to neurturin (SEQ ID NO:80) there is one additional potential O-linked glycosylation site one residue upstream from the first canonical framework cysteine (position 51 in the sequence as shown in Figure 11). In a mature persephin molecule based upon homology to GDNF there are five potential O-linked glycosylation sites in the 43 amino acids upstream from the first canonical framework cysteine at positions 9, 15, 18, 22 and 43 in SEQ ID NO:81 (corresponding to positions 17, 23, 26, 30 and 51 in the sequence as shown in Figure 11) along with the ten potential O-linked glycosylation sites noted above in the region between the canonical framework cysteines (corresponding to positions 48, 50, 62, 74, 81, 84, 105, 106, 111 and 126 in SEQ ID NO:81 and positions 56, 58, 70, 82, 89, 92, 113, 114, 119 and 134 in the sequence as shown in Figure 11).

Different possible cleavage sites may be present in the pre-pro-neurturin sequence. The amino acid sequence of the mature mouse neurturin (Figure 5, SEQ ID NO:2) is predicted from alignment with the N-terminal amino acid sequence of the purified chinese hamster neurturin. A four residue RRAR cleavage site (amino acids 92-95) is found immediately before the predicted N-terminal amino acid of mature mouse neurturin. This RRAR sequence fits the RXXR consensus sequence at which members of the TGF- $\beta$  superfamily are usually cleaved. This putative RRAR cleavage sequence is conserved in human neurturin. However, the mature human neurturin is predicted to have a two amino acid N-terminal extension relative to mature mouse neurturin when cleaved at this sequence. Since neurturin contains other sequences which fit the RXXR consensus (for example the sequence RRRR at amino acids 90-93) and the specificities of proteases involved in this cleavage are not completely understood, the possibility exists that in some situations, neurturin is cleaved at sites other than the above RRAR sequence, and the mature neurturin protein may have a variable number of amino acids preceding the cysteine residue at position 101 in the mouse sequence (pre-pro protein) and position 103 in the human sequence. Such alternate cleavage sites could be utilized differently among different organisms and among different tissues of the same organism. The N-terminal amino acids preceding the first of the seven conserved cysteines in the mature forms of members of the TGF- $\beta$  family vary greatly in both length and sequence. Furthermore, insertion of a ten amino acid sequence two residues upstream of the first conserved cysteine does not affect the known biological activities of one family member, dorsalin (Basler, K., Edlund, T., Jessell, T.M., and Yamada, T., (1993) Cell 73:687-702). Thus neurturin proteins which contain sequences of different lengths preceding the cysteine 101 in mouse and cysteine 103 in

human would be likely to retain their biological activity.

It is also believed that persephin proteins which contain sequences of different lengths preceding the first cysteine (residue No. 1 of mouse persephin in Figure 12 and residue No. 1 of rat persephin in Figure 14) would be likely to retain their biological activity.

The inventors herein believe that at a minimum the sequence of neurturin that will show biological activity will contain the sequence beginning at cysteine 103 and ending at cysteine 196 for human neurturin (Figure 7, SEQ ID NO:31) and beginning at cysteine 101 and ending at cysteine 194 for mouse neurturin (Figure 7, SEQ ID NO:32). Thus, within the scope of the neurturin polypeptides are amino acid sequences containing SEQ ID NO:31 and amino acid sequences containing SEQ ID NO:32 and nucleic acid sequences encoding these amino acid sequences.

Similarly, the inventors herein believe that, at a minimum, the sequence of persephin that will show biological activity will contain the sequence beginning at cysteine 1 and ending at cysteine 87 for mouse persephin (Figure 12, SEQ ID NO:79) and beginning at cysteine 1 and ending at cysteine 87 for rat persephin (Figure 14, SEQ ID NO:82). Thus, within the scope of persephin of the present invention are amino acid sequences containing SEQ ID NO:79 and amino acid sequences containing SEQ ID NO:82 and nucleic acid sequences encoding these amino acid sequences.

The present invention also encompasses nucleic acid sequences including sequences that encode mouse and rat persephin (Figures 11 and 14) as well as human persephin in the same manner that neurturin includes human and mouse neurturin nucleic acid sequences (Figures 7 and 8). Also included within the scope of this invention are sequences that are substantially the same as the nucleic

acid sequences encoding persephin or neurturin, respectively. Such substantially the same sequences may, for example, be substituted with codons more readily expressed in a given host cell such as *E. coli* according to well known and standard procedures. Such modified nucleic acid sequences are included within the scope of this invention.

Specific nucleic acid sequences can be modified by those skilled in the art and, thus, all nucleic acid sequences which encode for the amino acid sequences of pre-pro neurturin or persephin or the pre- region or the pro- region of neurturin or persephin can likewise be so modified. The present invention thus also includes nucleic acid sequence which will hybridize with all such nucleic acid sequences -- or complements of the nucleic acid sequences where appropriate -- and encode for a polypeptide having cell survival or growth promoting activity. The present invention also includes nucleic acid sequences which encode for polypeptides that have survival or growth promoting activity and that are recognized by antibodies that bind to neurturin or by antibodies that bind to persephin.

The present invention also encompasses vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the invention. This invention also includes host cells -- of any variety -- that have been transformed with vectors comprising expression regulatory elements operably linked to any of the nucleic acid sequences included within the scope of the present invention.

Methods are also provided herein for producing neurturin or persephin. Preparation can be by isolation from conditioned medium from a variety of cell types so long as the cell type produces neurturin or persephin. A second and preferred method involves utilization of

recombinant methods by isolating a nucleic acid sequence encoding neurturin or persephin, cloning the sequence along with appropriate regulatory sequences into suitable vectors and cell types, and expressing the sequence to  
5 produce neurturin or persephin.

A mammalian gene family comprised of four neurotrophic factors has been identified including nerve growth factor (NGF), brain derived neurotrophic factor (BDGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-  
10 4/5). These factors share approximately 60 percent nucleic acid sequence homology (Tuszynski and Gage, Ann Neurol 35:S9-S12, 1994 which is incorporated by reference). The persephin protein and the neurturin protein display no significant homology to the NGF family  
15 of neurotrophic factors. Either persephin or neurturin shares less than about 20% homology with the TGF- $\beta$  superfamily of growth factors. However, both persephin and neurturin show approximately 40% sequence identity with GDNF and approximately 50% sequence identity with  
20 each other. In particular, the positions of the seven cysteine residues present in persephin, neurturin and GDNF are nearly exactly conserved. The inventors herein believe that other unidentified genes may exist that encode proteins that have substantial amino acid sequence  
25 homology to persephin, neurturin and GDNF and which function as growth factors selective for the same or different tissues and the same or different biological activities and may act at the same or different receptors. A different spectrum of activity with respect  
30 to tissues affected and/or response elicited could result from preferential activation of different receptors by different family members as is known to occur with members of the NGF family of neurotrophic factors (Tuszynski and Gage, 1994, *supra*).

35 As a consequence of members of a particular gene family showing substantial conservation of amino acid



sequence among the protein products of the family members, there is considerable conservation of sequences at the DNA level. This forms the basis for a new approach for identifying other members of the gene family to which GDNF, neurturin and persephin belong. The method used for such identification is cross-hybridization using nucleic acid probes derived from one family member to form a stable hybrid duplex molecule with nucleic acid sequence from different members of the gene family or to amplify nucleic acid sequences from different family members. (see for example, Kaisho et al. *FEBS Letters* 266:187-191, 1990 which is incorporated by reference). The sequence from the different family member may not be identical to the probe, but will, nevertheless be sufficiently related to the probe sequence to hybridize with the probe. Alternatively, PCR using primers from one family member can be used to identify additional family members.

The above approaches have not heretofore been successful in identifying other gene family members because only one family member, GDNF was known. With the identification of neurturin in copending application Serial No. 08/519,777, however, unique new probes and primers can be made that contain sequences from the conserved regions of this gene family. The same conserved regions are also found in the third family member, persephin. In particular, three conserved regions have been identified herein which can be used as a basis for constructing new probes and primers. The new probes and primers made available from the work with neurturin and persephin make possible this powerful new approach which can now successfully identify other gene family members. Using this new approach, one may screen for genes related to GDNF, neurturin and persephin in sequence homology by preparing DNA or RNA probes based upon the conserved regions in the GDNF and neurturin

molecules. Therefore, one embodiment of the present invention comprises probes and primers that are unique to or derived from a nucleotide sequence encoding such conserved regions and a method for identifying further members of the neurturin-persephin-GDNF gene family.

Conserved-region amino acid sequences have been identified herein to include Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly-Tyr where Xaa<sub>1</sub> is Ser, Thr or Ala and Xaa<sub>2</sub> is Glu or Asp (SEQ ID NO:108); Glu-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Arg-Tyr-Cys-Xaa<sub>4</sub>-Gly-Xaa<sub>5</sub>-Cys in which Xaa<sub>1</sub> is Thr, Glu or lys, Xaa<sub>2</sub> is Val, Leu or Ile, Xaa<sub>3</sub> is Leu or Ile, Xaa<sub>4</sub> is Ala or Ser, and Xaa<sub>5</sub> is Ala or Ser, (SEQ ID NO:113); and Cys-Cys-Xaa<sub>1</sub>-Pro-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Xaa<sub>5</sub>-Asp-Xaa<sub>6</sub>-Xaa<sub>7</sub>-Xaa<sub>8</sub>-Phe-Leu-Asp-Xaa<sub>9</sub> in which Xaa<sub>1</sub> is Arg or Gln, Xaa<sub>2</sub> is Thr or Val or Ile, Xaa<sub>3</sub> is Ala or Ser, Xaa<sub>4</sub> is Tyr or Phe, Xaa<sub>5</sub> is Glu, Asp or Ala, Xaa<sub>6</sub> is Glu, Asp or no amino acid, Xaa<sub>7</sub> is val or leu, Xaa<sub>8</sub> is Ser or Thr, and Xaa<sub>9</sub> is Asp or Val (SEQ ID NO:114). Nucleotide sequences containing a coding sequence for the above conserved sequences or fragments of the above conserved sequences can be used as probes. Exemplary probe and primer sequences encoding amino acid sequences and SEQ ID NOS:125-129; primers whose reverse complementary sequences encode amino acid sequences SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:130; and, in particular, nucleotide sequences, SEQ ID NOS:115-124. Additional primers based upon GDNF and neurturin include nucleic acid sequences encoding amino acid sequences, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:40 and SEQ ID NO:41; primers whose reverse complementary sequences encode SEQ ID NO:37, SEQ ID NO:38 and SEQ ID NO 39; and, in particular, nucleic acid sequences, SEQ ID NOS:42-48.

Hybridization using the new probes from conserved regions of the nucleic acid sequences would be performed under reduced stringency conditions. Factors involved in determining stringency conditions are well known in the art (for example, see Sambrook et al., *Molecular Cloning*,

2nd Ed., 1989 which is incorporated by reference). Sources of nucleic acid for screening would include genomic DNA libraries from mammalian species or cDNA libraries constructed using RNA obtained from mammalian  
5 cells cloned into any suitable vector.

PCR primers would be utilized under PCR conditions of reduced annealing temperature which would allow amplification of sequences from gene family members other than GDNF, neurturin and persephin. Sources of nucleic  
10 acid for screening would include genomic DNA libraries from mammalian species cloned into any suitable vector, cDNA transcribed from RNA obtained from mammalian cells, and genomic DNA from mammalian species.

DNA sequences identified on the basis of  
15 hybridization or PCR assays would be sequenced and compared to GDNF, neurturin and persephin. The DNA sequences encoding the entire sequence of the novel factor would then be obtained in the same manner as described herein. Genomic DNA or libraries of genomic  
20 clones can also be used as templates because the intron/exon structures of GDNF and neurturin are conserved and coding sequences of the mature proteins are not interrupted by introns.

Using this approach as described above, the primers  
25 designed from the conserved regions of neurturin and GDNF have been used to identify and obtain the sequence of the new family member described herein, persephin. Degenerate primers designed from persephin, neurturin and GDNF can be further used to identify and obtain  
30 additional family members.

It is believed that all GDNF-neurturin-persephin family members will have a high degree of sequence identity with one or more of the three identified family-member consensus regions in the portion of the sequence  
35 between the first and seventh canonical framework cysteines (see Figure 12). In particular, a new family

member is anticipated to have at least a 62.5% identity with the consensus region octapeptide, Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly-Tyr where Xaa<sub>1</sub> is Ser, Thr or Ala and Xaa<sub>2</sub> is Glu or Asp (SEQ ID NO:108) or at least a 62.5 percent  
5 sequence identity with the consensus region octapeptide, Phe-Arg-Tyr-Cys-Xaa<sub>1</sub>-Gly-Xaa<sub>2</sub>-Cys where Xaa<sub>1</sub> and Xaa<sub>2</sub> are alanine or serine (SEQ ID NO:109) or at least a 50 percent sequence identity with the consensus region octapeptide, Asp-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Leu-Asp-Xaa<sub>4</sub>, where Xaa<sub>1</sub>  
10 is aspartic acid or glutamic acid or no amino acid, Xaa<sub>2</sub> is valine or leucine, Xaa<sub>3</sub> is serine or threonine; and Xaa<sub>4</sub> is valine or aspartic acid (SEQ ID NO:110). The inventors herein believed that any new family member will have 28 amino acids in the aligned sequence between the  
15 first and seventh canonical framework cysteine residues as set forth in Figure 15 with residues numbered from the N-terminal end of the family member aligned sequence being (1) Cys, (3) Leu, (10) Val, (13) Leu, (14) Gly, (15) Leu, (16) Gly, (17) Tyr, (21) Glu, (25) Phe, (26)  
20 Arg, (27) Tyr, (28) Cys, (30) Gly, (32) Cys, (44) Leu, (47) Leu, (58) Cys, (59) Cys (61) Pro, (66) Asp, (69) Phe, (70) Leu, (71) Asp, (83) Ser, (84) Ala, (87) Cys, and (89) Cys, however, it is possible that there may be as many as three mismatches.

25 Although neurturin has been purified on the basis of its ability to promote the survival of a particular neuronal type, this factor will act on other neuronal cell types as well. For example, neurturin is shown herein to promote the survival of nodose sensory ganglia  
30 neurons (see Example 3). Neurturin is also likely to promote the survival of non-neuronal cells. Indeed, all the growth factors isolated to date have been shown to act on many different cell types (for example see Scully and Otten, *Cell Biol Int* 19:459-469, 1994; Hefti,  
35 *Neurotrophic Factor Therapy* 25:1418-1435, 1994 which are incorporated by reference). It is known that NGF acts on

sympathetic neurons, several types of sensory neurons and certain populations of CNS neurons. GDNF, which is more closely related to neurturin, has been shown to act on dopaminergic, sympathetic, motor and several sensory neurons (Henderson et al. *supra*, 1994; Miles et al, *J Cell Biol* 130:137-148, 1995; Yan et al, *Nature* 373:341-344, 1995; Lin et al, *Science* 260:1130-1132, 1993; Trupp et al, *J Cell Biol* 130:137-148, 1995; Martin et al *Brain Res* 683:172-178, 1995; Bowenkamp et al *J Comp Neurol* 355:479-489, 1995 which are incorporated by reference). Thus, it is likely that in addition to peripheral sympathetic and sensory neurons, neurturin can act on a wide variety of central and peripheral neuronal cell types.

On the basis of the structural similarities of persephin to the sequences of neurturin and GDNF, persephin is also believed to promote the survival and growth of neuronal as well as non-neuronal cells. Indeed, as noted above, all the growth factors isolated to date have been shown to act on many different cell types (Scully and Otten, *Cell Biol Int* 19:459-469, 1995; Hefti, *Neurotrophic Factor Therapy* 25:1418-1435, 1994). Furthermore, the inventors herein have identified brain and heart tissues as tissues expressing persephin, which further supports the conclusion that persephin can act to promote survival and growth in a variety of neuronal and non-neuronal cells.

As an example of the actions of neurotrophic factors on non-neuronal tissues, the prototypical neurotrophic factor, NGF, also acts upon mast cells to increase their number when injected into newborn rats (Aloe, *J Neuroimmunol* 18:1-12, 1988). In addition, mast cells express the trk receptor and respond to NGF such that NGF is a mast cell secretagogue and survival promoting factor (Horigome et al., *J Biol Chem* 269:2695-2707, 1994 which is incorporated by reference). Moreover, members of the

TGF- $\beta$  superfamily act on many cell types of different function and embryologic origin.

The inventors herein have identified several non-neuronal tissues in which neurturin is expressed  
5 including blood, bone marrow, neonatal liver and mast cells. This suggests a role for neurturin in hematopoiesis, inflammation, allergy, and cardiomyopathy.

Similarly, the inventors herein have identified brain and heart as tissues in which persephin is  
10 expressed and it is further believed that persephin is expressed in a number of other neuronal and non-neuronal tissues. Thus, persephin may also have a role in hematopoiesis, inflammation, allergy and cardiomyopathies.

15       Neurotrophic factors of the NGF family are thought to act through factor-specific high affinity receptors (Tuszynski and Gage, 1994, *supra*). Only particular portions of the protein acting at a receptor site are required for binding to the receptor. Such particular  
20 portions or discrete fragments can serve as an agonist where the substance activates the receptor to elicit the promoting action on cell survival and growth and antagonists to neurturin or persephin where they bind to, but do not activate, the receptor or promote survival and  
25 growth. Such portions or fragments that are agonists and those that are antagonists are also within the scope of the present invention.

Synthetic, pan-growth factors can also be constructed by combining the active domains of persephin  
30 or neurturin with the active domains of one or more other growth factors. (For example, see Ilag et al., *Proc Nat'l Acad Sci* 92:607-611, 1995 which is incorporated by reference). These pan-growth factors would be expected to have the combined activities of neurturin or persephin  
35 and the one or more other growth factors. As such they are believed to be potent and multispecific growth

factors that are useful in the treatment of a wide spectrum of degenerative diseases and conditions including conditions that can be treated by any and all of the parent factors from which the active domains were  
5 obtained. Such pan-growth factors might also provide synergistic effects beyond the activities of the parent factors (Barres et al., *supra*).

Pan-growth factors within the scope of the present invention can also include chimeric or hybrid  
10 polypeptides that are constructed from portions of fragments of at least two growth factors. Growth factors of the TGF- $\beta$  superfamily are structurally related having highly conserved sequence landmarks whereby family members are identified. In particular, seven canonical  
15 framework cysteine residues are nearly invariant in members of the superfamily (Kingsley, *Genes & Dev* 8:133-146, 1994 which is incorporated by reference)(see Figure 17). Chimeric polypeptide molecules can, therefore, be constructed from a sequence that is substantially  
20 identical to a portion of either the persephin or the neurturin molecule, up to one or more crossover points, and one or more sequences each of which is substantially identical with a portion of another TGF- $\beta$  superfamily member extending on the other side of the corresponding  
25 one or more crossover points. For example, a portion of the amino terminal end of the persephin polypeptide can be combined with a portion of the carboxy terminal end of a neurturin polypeptide or alternatively a portion of the amino terminal end of a neurturin polypeptide can be  
30 combined with a portion of the carboxy terminal end of a persephin polypeptide. Such portions of neurturin or persephin polypeptides are preferably from about 5 to about 95, more preferably from about 10 to about 90, still more preferably from about 20 to about 80 and most  
35 preferably from about 30 to about 70 contiguous amino acids and such portions of another, non-persephin or, as

the case may be, non-neurturin TGF- $\beta$  superfamily member are preferably from about 5 to about 95, more preferably from about 10 to about 90, still more preferably from about 20 to about 80 and most preferably from about 30 to about 70 contiguous amino acids. For example, a particular crossover point might be between the third and fourth canonical framework cysteine residues. One such exemplary construct would contain at the 5' end a sequence comprised of a persephin sequence from residue 1 through the third canonical framework cysteine residue 37 and up to a cross-over point somewhere between residue 37 and residue 63 but not including the fourth canonical framework cysteine residue 64 (for reference, see mature persephin, SEQ ID NO:80). The 3' end of the hybrid construct would constitute a sequence derived from another TGF- $\beta$  superfamily member such as, for example, neurturin which is another TGF- $\beta$  superfamily member that is closely related to persephin. Using neurturin as the other TGF- $\beta$  family member, the hybrid construct beyond the crossover point would be comprised of a sequence beginning at the desired crossover point in the neurturin sequence between the third canonical framework cysteine residue 37 and the fourth canonical framework cysteine residue 67 of neurturin and continuing through residue 100 at the 3' end of neurturin (for alignment, see figure 12). A second exemplary hybrid construct would be comprised of residue 1 through a crossover point between residues 37 and 67 of neurturin contiguously linked with residues from the crossover point between residues 37 and 64 through residue 96 of persephin. The above constructs with persephin and neurturin are intended as examples only with the particular TGF- $\beta$  family member being selected from family members including but not limited to transforming growth factor- $\beta$ 1 (TGF $\beta$ 1), transforming growth factor- $\beta$ 2 (TGF $\beta$ 2), transforming growth factor- $\beta$ 3 (TGF $\beta$ 3), inhibin B A (INHSA), inhibin B B (INHBB), the



*nodal* gene (NODAL), bone morphogenetic proteins 2 and 4 (BMP2 and BMP4), the *Drosophila decapentaplegic* gene (*dpp*), bone morphogenetic proteins 5-8 (BMP5, BMP6, BMP7 and BMP8), the *Drosophila* 60A gene family (60A), bone morphogenetic protein 3 (BMP3), the *Vgl* gene, growth differentiation factors 1 and 3 (GDF1 and GDF3), dorsalin (*drsln*), inhibin  $\alpha$  (INH $\alpha$ ), the *MIS* gene (MIS), growth factor 9 (GDF-9), glial-derived neurotropic growth factor (GDNF), neurturin (NTN) and persephin (see Figure 16).

10 In addition, the crossover point can be any residue between the first and seventh canonical framework cysteines molecules of neurturin and the particular other family member. Furthermore, additional crossover points can be used to incorporate any desired number of

15 persephin portions or fragments with portions or fragments of any one or more other family members.

In constructing a particular chimeric molecule, the portions of persephin and portions of the other, non-persephin growth factor are amplified using PCR, mixed

20 and used as template for a PCR reaction using the forward primer from one and the reverse primer from the other of the two component portions of the chimeric molecule. Thus, for example a forward and reverse primers are selected to amplify the portion of persephin from the

25 beginning to the selected crossover point between the third and fourth canonical cysteine residues using a persephin plasmid as template. A forward primer with a 5' portion overlapping with the persephin sequence and a reverse primer are then used to amplify the portion of

30 the other, non-persephin growth factor member of the TGF- $\beta$  superfamily from the corresponding crossover point through the 3' end using a plasmid template containing the coding sequence for the non-persephin TGF- $\beta$  family member. The products of the two PCR reactions are gel

35 purified and mixed together and a PCR reaction performed. Using an aliquot of this reaction as template a PCR

reaction is performed using the persephin forward primer and the reverse primer for the non-persephin growth factor. The product is then cloned into an expression vector for production of the chimeric molecule.

5 Chimeric growth factors would be expected to be effective in promoting the growth and development of cells and for use in preventing the atrophy, degeneration or death of cells, particular in neurons. The chimeric polypeptides may also act as a receptor antagonists of  
10 one or both of the full length growth factors from which the chimeric polypeptide was constructed or as an antagonist of any other growth factor that acts at the same receptor or receptors.

The present invention also includes therapeutic or  
15 pharmaceutical compositions comprising persephin or neurturin in an effective amount for treating patients with cellular degeneration or dysfunction and a method comprising administering a therapeutically effective amount of neurturin or persephin. These compositions and  
20 methods are useful for treating a number of degenerative diseases. Where the cellular degeneration involves neuronal degeneration, the diseases include, but are not limited to peripheral neuropathy, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease,  
25 Huntington's disease, ischemic stroke, acute brain injury, acute spinal chord injury, nervous system tumors, multiple sclerosis, peripheral nerve trauma or injury, exposure to neurotoxins, metabolic diseases such as diabetes or renal dysfunctions and damage caused by  
30 infectious agents. In particular, the ability of persephin to promote survival in mesencephalic cells suggests an applicability of this growth factor in treating neuronal degenerative diseases of the CNS such as Parkinson's disease.

35 Where the cellular degeneration involves bone marrow cell degeneration, the diseases include, but are not

limited to disorders of insufficient blood cells such as, for example, leukopenias including eosinopenia and/or basopenia, lymphopenia, monocytopenia, neutropenia, anemias, thrombocytopenia as well as an insufficiency of stem cells for any of the above. The cellular degeneration can also involve myocardial muscle cells in diseases such as cardiomyopathy and congestive heart failure. The above cells and tissues can also be treated for depressed function.

10       The compositions and methods herein can also be useful to prevent degeneration and/or promote survival in other non-neuronal tissues as well. One skilled in the art can readily determine using a variety of assays known in the art for identifying whether neurturin or persephin  
15       would be useful in promoting survival or functioning in a particular cell type.

          In certain circumstances, it may be desirable to modulate or decrease the amount of persephin or neurturin expressed. Thus, in another aspect of the present  
20       invention, persephin or neurturin anti-sense oligonucleotides can be made and a method utilized for diminishing the level of expression of persephin or neurturin, respectively, by a cell comprising administering one or more persephin or neurturin anti-  
25       sense oligonucleotides. By persephin or neurturin anti-sense oligonucleotides reference is made to oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary nucleic acid sequence involved in the  
30       expression of persephin or neurturin, respectively, such that the expression of persephin or neurturin is reduced. Preferably, the specific nucleic acid sequence involved in the expression of persephin or neurturin is a genomic DNA molecule or mRNA molecule that contains sequences of  
35       the persephin or neurturin gene. This genomic DNA molecule can comprise flanking regions of the persephin

or neurturin gene, untranslated regions of persephin or neurturin mRNA, the pre- or pro- portions of the persephin or neurturin gene or the coding sequence for mature persephin or neurturin protein. The term

5 complementary to a nucleotide sequence in the context of persephin or neurturin antisense oligonucleotides and methods therefor means sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, i.e., under physiological conditions. The

10 persephin or neurturin antisense oligonucleotides preferably comprise a sequence containing from about 8 to about 100 nucleotides and more preferably the persephin or neurturin antisense oligonucleotides comprise from about 15 to about 30 nucleotides. The persephin or

15 neurturin antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages (Uhlmann and Peyman, *Chemical Reviews* 90:543-548, 1990; Schneider and Banner,

20 *Tetrahedron Lett* 31:335, 1990 which are incorporated by reference), modified nucleic acid bases and/or sugars and the like.

The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable

25 route known in the art including for example intravenous, subcutaneous, intramuscular, transdermal, intrathecal or intracerebral. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation. For treating

30 tissues in the central nervous system, administration can be by injection or infusion into the cerebrospinal fluid (CSF). When it is intended that neurturin or persephin be administered to cells in the central nervous system, administration can be with one or more agents capable of

35 promoting penetration of neurturin or persephin across the blood-brain barrier.

Persephin or neurturin can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, persephin or neurturin can be coupled to any substance known in the art to promote penetration or transport across the blood-brain barrier such as an antibody to the transferrin receptor, and administered by intravenous injection. (See for example, Friden et al., *Science* 259:373-377, 1993 which is incorporated by reference). Furthermore, persephin or neurturin can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. (See for example Davis et al. *Enzyme Eng* 4:169-73, 1978; Burnham, *Am J Hosp Pharm* 51:210-218, 1994 which are incorporated by reference).

The compositions are usually employed in the form of pharmaceutical preparations. Such preparations are made in a manner well known in the pharmaceutical art. One preferred preparation utilizes a vehicle of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous. Persephin or neurturin can also be incorporated into a solid or semi-solid biologically compatible matrix which can be implanted into tissues requiring treatment.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility,

stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or  
5 penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by  
10 continuous or periodic infusion.

Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

It is also contemplated that certain formulations  
15 containing persephin or neurturin are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose,  
20 sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and  
propylhydroxybenzoates, talc, magnesium, stearate, water,  
25 mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or  
30 delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation and promote absorption such as, for example, surface active  
35 agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Such calculations can be made without undue experimentation by one skilled in the art in light of the activity of neurturin or GDNF. With neurturin the activity in target cells data is disclosed herein and in copending application Serial Number 08/519,777 and in the case of persephin, the concentration required for activity at the cellular level is believed to be similar to that of neurturin. Persephin activity on a particular target cell type can be determined by routine experimentation. Exact dosages are determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration.

In one embodiment of this invention, persephin or neurturin may be therapeutically administered by implanting into patients vectors or cells capable of producing a biologically-active form of persephin or neurturin or a precursor of persephin or neurturin, i.e. a molecule that can be readily converted to a biological-active form of neurturin by the body. In one approach cells that secrete persephin or neurturin may be encapsulated into semipermeable membranes for implantation into a patient. The cells can be cells that

normally express persephin or neurturin or a precursor thereof or the cells can be transformed to express persephin or neurturin or a precursor thereof. It is preferred that the cell be of human origin and that the  
5 persephin or neurturin be human persephin or neurturin when the patient is human. However, the formulations and methods herein can be used for veterinary as well as human applications and the term "patient" as used herein is intended to include human and veterinary patients.

10 Cells can be grown *ex vivo* for use in transplantation or engraftment into patients (Muench et al., *Leuk & Lymph* 16:1-11, 1994 which is incorporated by reference). In another embodiment of the present invention, persephin or neurturin is used to promote the  
15 *ex vivo* expansion of a cells for transplantation or engraftment. Current methods have used bioreactor culture systems containing factors such as erythropoietin, colony stimulating factors, stem cell factor, and interleukins to expand hematopoietic  
20 progenitor cells for erythrocytes, monocytes, neutrophils, and lymphocytes (Verfaillie, *Stem Cells* 12:466-476, 1994 which is incorporated by reference). These stem cells can be isolated from the marrow of human donors, from human peripheral blood, or from umbilical  
25 cord blood cells. The expanded blood cells are used to treat patients who lack these cells as a result of specific disease conditions or as a result of high dose chemotherapy for treatment of malignancy (George, *Stem Cells* 12(Suppl 1):249-255, 1994 which is incorporated by  
30 reference). In the case of cell transplant after chemotherapy, autologous transplants can be performed by removing bone marrow cells before chemotherapy, expanding the cells *ex vivo* using methods that also function to purge malignant cells, and transplanting the expanded  
35 cells back into the patient following chemotherapy (for review see Rummel and Van Zant, *J Hematotherapy* 3:213-



218, 1994 which is incorporated by reference). Since persephin or neurturin is believed to be expressed in the developing animal in blood, bone marrow and liver, tissues where proliferation and differentiation of progenitor cells occur, it is believed that persephin or neurturin can function to regulate the proliferation of hematopoietic stem cells and the differentiation of mature hematopoietic cells. Thus, the addition of persephin or neurturin to culture systems used for ex vivo expansion of cells could stimulate the rate at which certain populations of cells multiply or differentiate, and improve the effectiveness of these expansion systems in generating cells needed for transplant.

It is also believed that persephin or neurturin can be used for the ex vivo expansion of precursor cells in the nervous system. Transplant or engraftment of cells is currently being explored as a therapy for diseases in which certain populations of neurons are lost due to degeneration such as, for example, in parkinson's disease (Bjorklund, *Curr Opin Neurobiol* 2:683-689, 1992 which is incorporated by reference). Neuronal precursor cells can be obtained from animal or human donors or from human fetal tissue and then expanded in culture using persephin or neurturin or other growth factors. These cells can then be engrafted into patients where they would function to replace some of the cells lost due to degeneration. Because neurotrophins have been shown to be capable of stimulating the survival and proliferation of neuronal precursors cells such as, for example, NT-3 stimulation of sympathetic neuroblast cells (Birren et al., *Develop* 119:597-610, 1993 which is incorporated by reference), persephin or neurturin could also function in similar ways during the development of the nervous system and could be useful in the ex vivo expansion of neuronal cells.

In a number of circumstances it would be desirable to determine the levels of persephin or neurturin in a patient. The identification of persephin or neurturin along with the present report that persephin and  
5 neurturin are expressed by a number of tissues provides the basis for the conclusion that the presence of persephin or neurturin serves a normal physiologic function related to cell growth and survival. Indeed, other neurotrophic factors are known to play a role in  
10 the function of neuronal and non-neuronal tissues. (For review see Scully and Otten, *Cell Biol Int* 19:459-469, 1995; Otten and Gadiant, *Int J Devl Neurosciences* 13:147-151, 1995 which are incorporated by reference). Endogenously produced persephin or neurturin may also  
15 play a role in certain disease conditions, particularly where there is cellular degeneration such as in neurodegenerative conditions or diseases. Other neurotrophic factors are known to change during disease conditions. For example, in multiple sclerosis, levels  
20 of NGF protein in the cerebrospinal fluid are increased during acute phases of the disease (Bracci-Laudiero et al., *Neuroscience Lett* 147:9-12, 1992 which is incorporated by reference) and in systemic lupus erythematosus there is a correlation between inflammatory  
25 episodes and NGF levels in sera (Bracci-Laudiero et al. *NeuroReport* 4:563-565, 1993 which is incorporated by reference).

Given that neurturin is expressed in blood cells, bone marrow and mast cells, and it is believed that  
30 persephin is similarly expressed, it is likely that the level of persephin or neurturin may be altered in a variety of conditions and that quantification of persephin or neurturin levels would provide clinically useful information. Furthermore, in the treatment of  
35 degenerative conditions, compositions containing either persephin or neurturin or both can be administered and it

would likely be desirable to achieve certain target levels of persephin and/or neurturin, as the case may be, in sera, in cerebrospinal fluid or in any desired tissue compartment. It would, therefore, be advantageous to be  
5 able to monitor the levels of the particular growth factor, persephin or neurturin, in a patient. Accordingly, the present invention also provides methods for detecting the presence of persephin or for detecting the presence of neurturin in a sample from a patient.

10 The term "detection" as used herein in the context of detecting the presence of persephin or neurturin in a patient is intended to include the determining of the amount of persephin or neurturin or the ability to express an amount of persephin or neurturin in a patient,  
15 the distinguishing of persephin or neurturin from other growth factors, the estimation of prognosis in terms of probable outcome of a degenerative disease and prospect for recovery, the monitoring of the persephin or neurturin levels over a period of time as a measure of  
20 status of the condition, and the monitoring of persephin or neurturin levels for determining a preferred therapeutic regimen for the patient.

To detect the presence of persephin or neurturin in a patient, a sample is obtained from the patient. The  
25 sample can be a tissue biopsy sample or a sample of blood, plasma, serum, CSF or the like. Neurturin is expressed in a wide variety of tissues as shown in example 9 and it is believed that persephin as well is secreted in a number of tissues. Thus, samples for  
30 detecting persephin or neurturin can be taken from any tissues expressing the particular growth factor. When assessing peripheral levels of persephin or neurturin, it is preferred that the sample be a sample of blood, plasma or serum. When assessing the levels of persephin or  
35 neurturin in the central nervous system a preferred sample is a sample obtained from cerebrospinal fluid.

In some instances it is desirable to determine whether the persephin or neurturin gene is intact in the patient or in a tissue or cell line within the patient. By an intact persephin or neurturin gene it is meant that there are no alterations in the gene such as point mutations, deletions, insertions, chromosomal breakage, chromosomal rearrangements and the like wherein such alteration might alter production of persephin or neurturin or alter its biological activity, stability or the like to lead to disease processes or susceptibility to cellular degenerative conditions. Conversely, by a non-intact persephin or neurturin gene it is meant that such alterations are present. Thus, in one embodiment of the present invention a method is provided for detecting and characterizing any alterations in the persephin or neurturin gene. The method comprises providing an oligonucleotide that contains the persephin or neurturin cDNA, genomic DNA or a fragment thereof or a derivative thereof. By a derivative of an oligonucleotide, it is meant that the derived oligonucleotide is substantially the same as the sequence from which it is derived in that the derived sequence has sufficient sequence complementarily to the sequence from which it is derived to hybridize to the persephin or neurturin gene. The derived nucleotide sequence is not necessarily physically derived from the nucleotide sequence, but may be generated in any manner including for example, chemical synthesis or DNA replication or reverse transcription or transcription.

Typically, patient genomic DNA is isolated from a cell sample from the patient and digested with one or more restriction endonucleases such as, for example, TaqI and AluI. Using the Southern blot protocol, which is well known in the art, this assay determines whether a patient or a particular tissue in a patient has an intact

persephin or neurturin gene or a persephin or neurturin gene abnormality.

Hybridization to the persephin or neurturin gene would involve denaturing the chromosomal DNA to obtain a single-stranded DNA; contacting the single-stranded DNA with a gene probe associated with the persephin or neurturin gene sequence; and identifying the hybridized DNA-probe to detect chromosomal DNA containing at least a portion of the human persephin or neurturin gene.

10       The term "probe" as used herein refers to a structure comprised of a polynucleotide which forms a hybrid structure with a target sequence, due to complementarity of probe sequence with a sequence in the target region. Oligomers suitable for use as probes may  
15       contain a minimum of about 8-12 contiguous nucleotides which are complementary to the targeted sequence and preferably a minimum of about 20.

      The persephin or neurturin gene probes of the present invention can be DNA or RNA oligonucleotides and  
20       can be made by any method known in the art such as, for example, excision, transcription or chemical synthesis. Probes may be labelled with any detectable label known in the art such as, for example, radioactive or fluorescent labels or enzymatic marker. Labeling of the probe can be  
25       accomplished by any method known in the art such as by PCR, random priming, end labelling, nick translation or the like. One skilled in the art will also recognize that other methods not employing a labelled probe can be used to determine the hybridization. Examples of methods  
30       that can be used for detecting hybridization include Southern blotting, fluorescence *in situ* hybridization, and single-strand conformation polymorphism with PCR amplification.

      Hybridization is typically carried out at 25-45°C,  
35       more preferably at 32-40°C and more preferably at 37-38°C. The time required for hybridization is from about

0.25 to about 96 hours, more preferably from about one to about 72 hours, and most preferably from about 4 to about 24 hours.

Persephin or neurturin gene abnormalities can also  
5 be detected by using the PCR method and primers that flank or lie within the persephin or neurturin gene. The PCR method is well known in the art. Briefly, this method is performed using two oligonucleotide primers which are capable of hybridizing to the nucleic acid  
10 sequences flanking a target sequence that lies within a persephin or neurturin gene and amplifying the target sequence. The terms "oligonucleotide primer" as used herein refers to a short strand of DNA or RNA ranging in length from about 8 to about 30 bases. The upstream and  
15 downstream primers are typically from about 20 to about 30 base pairs in length and hybridize to the flanking regions for replication of the nucleotide sequence. The polymerization is catalyzed by a DNA-polymerase in the presence of deoxynucleotide triphosphates or nucleotide  
20 analogs to produce double-stranded DNA molecules. The double strands are then separated by any denaturing method including physical, chemical or enzymatic. Commonly, the method of physical denaturation is used involving heating the nucleic acid, typically to  
25 temperatures from about 80°C to 105°C for times ranging from about 1 to about 10 minutes. The process is repeated for the desired number of cycles.

The primers are selected to be substantially complementary to the strand of DNA being amplified.  
30 Therefore, the primers need not reflect the exact sequence of the template, but must be sufficiently complementary to selectively hybridize with the strand being amplified.

After PCR amplification, the DNA sequence comprising  
35 persephin or neurturin or pre-pro persephin or neurturin or a fragment thereof is then directly sequenced and

analyzed by comparison of the sequence with the sequences disclosed herein to identify alterations which might change activity or expression levels or the like.

In another embodiment a method for detecting  
5 persephin or neurturin is provided based upon an analysis of tissue expressing the persephin gene or the neurturin gene. Certain tissues such as those identified below in example 9 have been found to express the neurturin gene. It is also believed that a number of tissues will express  
10 the persephin gene based upon the observations for neurturin and the identification herein of brain and heart as tissues expressing persephin. The method comprises hybridizing a polynucleotide to mRNA from a sample of tissues that normally express the persephin  
15 gene or the neurturin gene. The sample is obtained from a patient suspected of having an abnormality in the persephin gene or the neurturin gene or in the persephin gene or the neurturin gene of particular cells. In the case of neurturin, the polynucleotide comprises SEQ ID  
20 NO:11 or a derivative thereof or a fragment thereof. In the case of persephin, the polynucleotide comprises SEQ ID NO:105 or SEQ ID NO:107 or the human ortholog of persephin or derivatives thereof or fragments thereof.

To detect the presence of mRNA encoding persephin  
25 protein or neurturin protein, a sample is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis  
30 or other size separation techniques.

The mRNA of the sample is contacted with a DNA sequence serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

35 When using the cDNA encoding persephin protein or neurturin protein or a derivative of the cDNA as a probe,

high stringency conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of persephin or neurturin nucleotide sequences when in fact an intact and functioning  
5 persephin gene or neurturin gene is not present. When using sequences derived from the persephin or neurturin cDNA, less stringent conditions could be used, however, this would be a less preferred approach because of the likelihood of false positives. The stringency of  
10 hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook et al. (Sambrook, et al., 1989,  
15 supra).

In order to increase the sensitivity of the detection in a sample of mRNA encoding the persephin protein or neurturin protein, the technique of reverse transcription/polymerization chain reaction (RT/PCR) can  
20 be used to amplify cDNA transcribed from mRNA encoding the persephin protein or the neurturin protein. The method of RT/PCR is well known in the art (see example 9 and figure 6 below).

The RT/PCR method can be performed as follows.  
25 Total cellular RNA is isolated by, for example, the standard guanidium isothiocyanate method and the total RNA is reverse transcribed. The reverse transcription method involves synthesis of DNA on a template of RNA using a reverse transcriptase enzyme and a 3' end primer.  
30 Typically, the primer contains an oligo(dT) sequence. The cDNA thus produced is then amplified using the PCR method and persephin specific primers or neurturin specific primers. (Belyavsky et al, *Nucl Acid Res* 17:2919-2932, 1989; Krug and Berger, *Methods in*  
35 *Enzymology*, Academic Press, N.Y., Vol.152, pp. 316-325, 1987 which are incorporated by reference).



The polymerase chain reaction method is performed as described above using two oligonucleotide primers that are substantially complementary to the two flanking regions of the DNA segment to be amplified.

5       Following amplification, the PCR product is then electrophoresed and detected by ethidium bromide staining or by phosphoimaging.

The present invention further provides for methods to detect the presence of the persephin protein or the  
10   neurturin protein in a sample obtained from a patient. Any method known in the art for detecting proteins can be used. Such methods include, but are not limited to immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical  
15   techniques, agglutination and complement assays. (for example see *Basic and Clinical Immunology*, Sites and Terr, eds., Appleton & Lange, Norwalk, Conn. pp 217-262, 1991 which is incorporated by reference). Preferred are binder-ligand immunoassay methods including reacting  
20   antibodies with an epitope or epitopes of the persephin protein or reacting antibodies with an epitope or epitopes of the neurturin protein and competitively displacing a labeled persephin protein or a labeled neurturin protein or derivative thereof.

25       As used herein, a derivative of the persephin protein or a derivative of the neurturin protein is intended to include a polypeptide in which certain amino acids have been deleted or replaced or changed to modified or unusual amino acids wherein the persephin  
30   derivative or the neurturin derivative is biologically equivalent to persephin or neurturin, respectively, and wherein the polypeptide derivative cross-reacts with antibodies raised against the persephin protein or the neurturin protein, respectively. By cross-reaction it is  
35   meant that an antibody reacts with an antigen other than the one that induced its formation.

Numerous competitive and non-competitive protein binding immunoassays are well known in the art. Antibodies employed in such assays may be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemilumescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA), enzyme immunoassays, e.g., enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like.

Polyclonal or monoclonal antibodies to the persephin protein and to the neurturin protein or an epitope thereof can be made for use in immunoassays by any of a number of methods known in the art. By epitope reference is made to an antigenic determinant of a polypeptide. An epitope could comprise 3 amino acids in a spacial conformation which is unique to the epitope. Generally an epitope consists of at least 5 such amino acids. Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and 2 dimensional nuclear magnetic resonance.

One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesizing the sequence and injecting it into an appropriate animal, usually a rabbit or a mouse (See Example 10).

Oligopeptides can be selected as candidates for the production of an antibody to the persephin protein or for the production of an antibody to the neurturin protein based upon the oligopeptides lying in hydrophilic regions, which are thus likely to be exposed in the mature protein.

Antibodies to persephin or to neurturin can also be raised against oligopeptides that include one or more of the conserved regions identified herein such that the antibody can cross-react with other family members. Such  
5 antibodies can be used to identify and isolate the other family members.

Methods for preparation of the persephin protein or to the neurturin protein or an epitope thereof include, but are not limited to chemical synthesis, recombinant  
10 DNA techniques or isolation from biological samples. Chemical synthesis of a peptide can be performed, for example, by the classical Merrifield method of solid phase peptide synthesis (Merrifield, *J Am Chem Soc* 85:2149, 1963 which is incorporated by reference) or the FMOC strategy  
15 on a Rapid Automated Multiple Peptide Synthesis system (DuPont Company, Wilmington, DE) (Caprino and Han, *J Org Chem* 37:3404, 1972 which is incorporated by reference).

Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by  
20 subsequent boosts at appropriate intervals. The animals are bled and sera assayed against purified persephin protein or purified neurturin protein usually by ELISA or by bioassay based upon the ability to block the action of persephin or neurturin, as the case may be. When using  
25 avian species, e.g. chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared after the method of Milstein and Kohler by fusing splenocytes from immunized mice with continuously replicating tumor cells such as  
30 myeloma or lymphoma cells. (Milstein and Kohler *Nature* 256:495-497, 1975; Gutfre and Milstein, *Methods in Enzymology: Immunochemical Techniques* 73:1-46, Langone and Banatis eds., Academic Press, 1981 which are incorporated by reference). The hybridoma cells so  
35 formed are then cloned by limiting dilution methods and

supernates assayed for antibody production by ELISA, RIA or bioassay.

The unique ability of antibodies to recognize and specifically bind to target proteins provides an approach  
5 for treating an over expression of the protein. Thus, another aspect of the present invention provides for a method for preventing or treating diseases involving over expression of the persephin protein or the neurturin protein by treatment of a patient with specific  
10 antibodies to the persephin protein or to the neurturin protein, respectively.

Specific antibodies, either polyclonal or monoclonal, to the persephin protein or to the neurturin protein can be produced by any suitable method known in  
15 the art as discussed above. For example, murine or human monoclonal antibodies can be produced by hybridoma technology or, alternatively, the persephin protein or the neurturin protein, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or  
20 fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to the persephin protein or to the neurturin protein. Such antibodies can be from any class of antibodies including, but not limited to IgG,  
25 IgA, IgM, IgD, and IgE or in the case of avian species, IgY and from any subclass of antibodies.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one  
30 skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims  
35 which follow the examples.

## Example 1

This example illustrates the isolation and purification of neurturin from CHO cell conditioned medium.

5 Preparation of CHO cell conditioned medium:

A derivative of DG44 Chinese hamster ovary cells, DG44CHO-pHSP-NGFI-B (CHO) cells, was used (Day et al, *J Biol Chem* 265:15253-15260, 1990 which is incorporated by reference). As noted above, the inventors have also  
10 obtained neurturin in partially purified form from other derivatives of DG44 Chinese hamster ovary cells. The CHO cells were maintained in 20 ml medium containing minimum essential medium (MEM) alpha (Gibco-BRL No. 12561, Gaithersburg, MD) containing 10% fetal calf serum  
15 (Hyclone Laboratories, Logan, UT), 2 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 25nM methotrexate using 150 cm<sup>2</sup> flasks (Corning Inc., Corning NY). For passage and expansion, medium from a confluent flask was aspirated; the cells were washed with 10 ml  
20 phosphate buffered saline (PBS) containing in g/l, 0.144 KH<sub>2</sub>PO<sub>4</sub>, 0.795 Na<sub>2</sub>HPO<sub>4</sub> and 9.00 NaCl; and the flask was then incubated for 2-3 minutes with 2 ml 0.25% trypsin in PBS. Cells were then knocked off the flask surface, 8 ml of medium were added and cells were trituated several times  
25 with a pipette. The cells were split 1:5 or 1:10, incubated at 37°C under an atmosphere of 5% CO<sub>2</sub> in air and grown to confluence for 3-4 days.

The cell culture was then expanded into 850 cm<sup>2</sup> roller bottles (Becton Dickinson, Bedford, MA). A  
30 confluent 150 cm<sup>2</sup> flask was trypsinized and seeded into one roller bottle containing 240 ml of the above modified MEM medium without methotrexate. The pH was maintained either by blanketing the medium with 5% CO<sub>2</sub> in air or by preparing the medium with 25 mM HEPES pH 7.4 (Sigma, St.  
35 Louis, MO). The roller bottles were rotated at 0.8-1.0

revolutions per minute. Cells reached confluence in 4 days.

For collecting conditioned medium, serum-free CHO cell (SF-CHO) medium was used. SF-CHO was prepared using 1:1 DME/F12 base medium, which was prepared by mixing 1:1 (v/v) DMEM (Gibco-BRL product No. 11965, Gibco-BRL, Gaithersburg, MD) with Ham's F12 (Gibco-BRL product No. 11765). The final SF-CHO medium contained 15 mM HEPES pH 7.4 (Sigma, St. Louis, MO), 0.5 mg/ml bovine serum albumin (BSA, Sigma, St. Louis MO), 25 µg/ml heparin, (Sigma, St. Louis, MO), 1X insulin-transferrin-selenite supplement (bovine insulin, 5 µg/ml; human transferrin, 5 µg/ml; sodium selenite, 5 ng/ml; Sigma, St. Louis, MO), 2 mM l-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. The medium from the confluent roller bottles was removed and the cells washed once with 30 ml SF-CHO medium to remove serum proteins. Cells were then incubated at 37°C for 16-24 hrs in 80 ml SF-CHO medium to further remove serum proteins. The 80 ml medium was removed and discarded. A volume of 120 ml of SF-CHO medium was added to the flask and the cells incubated at 37°C. Every 48 hrs thereafter, 120 ml was collected and replaced with the same volume of SF-CHO medium.

Collected media was pooled and centrifuged at 4°C in polypropylene conical tubes to remove cellular debris and the supernatant stored at -70°C. Media was collected 5 times over 10 days to yield a total of approximately 600 ml conditioned medium per roller bottle.

Fractions collected from the columns at each stage of purification were assayed for biological activity using the neuronal survival assay and for protein content by the dye binding assay of Bradford (*Anal Biochem* 72:248 et seq., 1976 which is incorporated by reference). The total mg of protein in the starting volume, typically 50 liters, of conditioned medium was determined.

Superior Cervical Ganglion Survival Assay:

- The neurotrophic activity of CHO conditioned medium starting material and at various stages of purification was assessed using the superior cervical ganglion survival assay system previously reported (Martin, et al *J of Cell Biology* 106:829-844; Deckwerth and Johnson, *J Cell Bio* 123:1207-1222, 1993 which are incorporated by reference). Primary cultures of sympathetic neurons from superior cervical ganglion (SCG) were prepared by
- 5 dissecting tissue from Day 20-21 rat embryo (E20-E21). The SCG's were placed in Leibovitz's L15 with l-glutamine medium (Cat #11415-023 Gibco-BRL, Gaithersburg, MD), digested for 30 minutes with 1 mg/ml collagenase (Cat #4188 Worthington Biochemical, Freehold, NJ) in
  - 10 Leibovitz's L15 medium at 37°C, followed by a 30 minute digestion in trypsin-lyophilized & irradiated (Type TRLVMF Cat #4454 Worthington Biochemical, Freehold, NJ) which was resuspended in modified Hanks' Balanced Salt Solution (Cat #H-8389 Sigma Chemical Co., St. Louis, MO).
  - 15 The digestion was stopped using AM50 which contains Minimum Essential Medium with Earle's salts and without l-glutamine (Cat #11090-016 Gibco-BRL), 10% fetal calf serum (Cat #1115 Hyclone Laboratories, Logan, UT), 2mM l-glutamine (Cat #G5763 Sigma Chemical Co., St. Louis, MO),
  - 20 20 µM FuDr (F-0503 Sigma Chemical Co., St. Louis, MO), 20 µM Uridine (Cat #3003 Sigma Chemical Co., St. Louis, MO), 100 U/ml penicillin, 100 µg/ml Streptomycin, and 50 ng/ml 2.5 S NGF. The cells were dissociated into a suspension of single cells using a silanized and flame-polished
  - 25 Pasteur pipet. After filtration of the suspension through a nitex filter (size 3-20/14, Tetko Inc., Elmsford, NY), the cells were placed in AM50 medium as above and preplated on a 100 mm Falcon or Primaria culture dish (Becton Dickinson Labware, Lincoln Park, NJ)
  - 30 to reduce the number of non-neuronal cells. After 2 hours, the medium containing the unattached neuronal

cells was removed from these dishes and triturated again through a silanized and flame-polished Pasteur pipet. The single cell suspension was plated on 24-well tissue culture plates (Costar, Wilmington, MA) that have been previously coated with a double layer of collagen, one layer of collagen that had been ammoniated and a second layer of collagen that had been air dried. They were allowed to attach for 30 minutes to 2 hours. A specific number of viable cells, usually about 1200 to about 3000 total cells per well, or a specific percentage of the ganglion, usually 25% of the cells obtained per ganglion were plated into each well. When cell counts were to be performed they were placed in the 24-well dishes as stated above or alternatively, on 2-well chamber slides (Nunc, Naperville, IL). Cultures were then incubated for 5-6 days at 37° in AM50 medium in a 5% CO<sub>2</sub>/95% air atmosphere. The death of the cultured neurons was induced by exchanging the medium with medium without NGF and with 0.05% goat anti-NGF (final titer in the wells is 1:10). This NGF-deprivation results in death of the neurons over a period of 24-72 hours. Aliquots of partially purified or purified factor, or appropriate controls, were added to the cultures at the time of NGF removal to determine the ability to prevent the neuronal death.

Evaluation of the ability of column fraction, gel eluates, or purified factor to prevent neuronal death was by visual inspection of cultures under phase contrast microscopy. Viable neurons remained phase bright with intact neurites, whereas dead neurons were shrunken, phase dark, had irregular membranes and neurites were fragmented (Figure 3). Where precise quantitation of neuronal survival was required, the cultures were fixed in 4% paraformaldehyde or 10% Formalin in PBS, and stained with crystal violet solution, (Huntoon Formula Harleco E.M. Diagnostics Systems, Gibbstown, NJ). When



using 24 well dishes, 1  $\mu$ l crystal violet solution was added to each well containing 10% formalin and the cells were counted using a phase contrast microscope. If the 2-well chamber slides were used, the cultures were fixed, stained with crystal violet, destained with water, dehydrated in increasing ethanol concentrations to toluene, and mounted in a toluene-based mounting solution. Neurons were scored as viable if they had a clear nucleolus and nuclei and were clearly stained with crystal violet.

The neuronal death at 72 hours is shown in Figure 3B. Also shown are (A) the positive control cells maintained with nerve growth factor and (C) the cells treated with anti-NGF and neurturin (approximately 3 ng/ml) showing survival of neurons.

Activity was quantitated by calculation of a "survival unit". The total survival units in a sample were defined as the minimal volume of an aliquot of the sample which produced maximal survival divided into the total volume of that sample. Specific activity was calculated as the survival units divided by the mg total protein.

Survival units were determined in an assay using approximately 1200 viable neurons in a 0.5 ml culture assay and a culture period of 48 hours following addition of the fraction. Survival was assessed visually after the 48 hours. Intrinsic activity as shown in Figure 4 was determined in an assay using approximately 2700 neurons and a culture period of 72 hours. Survival was assessed by fixing the neurons and counting the number of surviving neurons. Because the stability, as assessed by half-life of activity, for neurturin decreases as the number of neurons increases, the intrinsic activity measurement would be expected to be lower than that predicted by Specific Activity determinations. The intrinsic activity measurement would also be expected to

be lower than that predicted by specific activity because the survival was measured after 72 hours instead of 48 hours.

To ensure the reproducibility of these activity unit assays, it was necessary to plate the primary neuronal cultures at reproducible cell densities, as the stability of the activity decreases significantly with increasing neuronal density. The range of cell densities was from about 1200 to about 2700 cells per well. The presence of soluble heparin in the assay medium had no effect on the short-term (~3 days) stability of the survival activity.

Purification of Neurturin:

Pooled conditioned medium was filtered through 0.2 µl pore bottle-top filters (cellulose acetate membrane, Corning Inc., Corning, NY). Typically 50 liters of conditioned medium was used and processed in 25 liter batches. Each 25 liter batch was introduced at a rate of 20 ml/min onto a 5 x 5 cm column containing 100 ml heparin-agarose (Sigma, St. Louis, MO) equilibrated with 25 mM HEPES, pH 7.4 buffer with 150 mM NaCl. The column was then washed with approximately 1000 ml 25 mM HEPES, pH 7.4 buffer containing 0.5 M NaCl at 20 ml/min and the activity was then eluted with 25 mM HEPES, pH 7.4 buffer containing 1.0 M NaCl. After switching to the 1.0M NaCl elution buffer, the first 50 ml of buffer was discarded and, thereafter, one 300 ml fraction was collected.

Pooled material eluted from the Heparin-agarose column was then diluted 1:1 (v/v) with 25 mM HEPES, pH 7.4 buffer containing 0.04% TWEEN 20 to a NaCl concentration of 0.5 M and introduced into a 1.5 cm x 9 cm column containing 16 ml SP SEPHAROSE® High Performance ion exchange resin (Pharmacia, Piscataway, NJ) equilibrated in 25 mM HEPES 7.4 containing 0.5 M NaCl and 0.02% TWEEN 20. The column was then washed with 160 ml 25 mM HEPES, pH 7.4 buffer containing 0.5 M NaCl and 0.02% TWEEN 20 and the activity was eluted with 25 mM

HEPES, pH 7.4 buffer containing 1.0 M NaCl and 0.02% TWEEN 20 at a flow rate of 2 ml/min. One 50 ml fraction was collected after the first 7 ml of eluate from the column.

- 5        Material eluted from the SP SEPHAROSE® column was fractionated using fast protein liquid chromatography (FPLC) on a Chelating Superose HR 10/2 column charged with Cu<sup>++</sup> (Pharmacia, Piscataway, NJ). The column had been prepared by washing with 10 ml water, charging with  
10    3 ml of 2.5 mg/ml CuSO<sub>4</sub>·5H<sub>2</sub>O, washing with 10 ml water, and equilibrating with 10 ml of 25 mM HEPES pH 7.4 buffer containing 1.0 M NaCl and 0.02% TWEEN 20. The eluate was introduced into the column in 25 mM HEPES, pH 7.4 buffer containing 1.0 M NaCl at a rate of 1.0 ml/min. The bound  
15    proteins were eluted with a linear gradient of increasing glycine concentration (0-300 mM) in 25 mM HEPES, pH 7.4 buffer containing 1.0 M NaCl at a rate of 1.0 ml/min. The gradient was produced by a Pharmacia FPLC system using an LCC-500 controller and P-500 pumps to establish  
20    a 0-300 mM glycine gradient in 40 ml at 1.0 ml/min, thus increasing the gradient by 7.5 mM glucine per min. One ml fractions were collected and assayed for SCG survival promotion. Peak activity was observed in fractions 17-20, i.e. 17-20 min or ml from the start of the gradient.
- 25        Absorbance measurements at 280 nM by an in-line UV monitor indicated that most proteins eluted prior to the survival activity in fractions 17-20. Thus, significant purification was achieved at this step. A 25 kD band co-purified with the survival activity.
- 30        The combined eluted fractions from the Cu<sup>++</sup> superose column were diluted to 0.45 M NaCl using 25 mM HEPES pH 7.4 buffer containing 0.02% TWEEN 20 and introduced into a Mono S HR 5/5 cation exchange column (Pharmacia, Piscataway, NJ) for further FPLC purification. The  
35    column had been equilibrated with 25 mM HEPES pH 7.4 buffer containing 0.45 M NaCl containing 0.02% TWEEN 20.

Bound proteins were eluted with a linear gradient of increasing NaCl concentration (0.45-1.0 M). The gradient was produced as described above from 0.45 M - 1.0 M NaCl in 35 mls at 1.0 ml/min, thus increasing concentration at 5 0.0157 M per ml or min. Thirteen 1.0 ml fractions (fractions 1-13) were collected followed by 44 0.5 ml fractions (fractions 14-53). Peak activity in SCG assay was in fractions 26-29. Each fraction was assayed in the SCG survival assay over a range of volumes of from 0.1 to 10 1.0  $\mu$ l per 0.5 ml culture medium.

One percent (5  $\mu$ l) of each fraction was loaded onto a non-reducing, 14% SDS polyacrylamide gel and electrophoresed for 750 V-hr at 25°C. Proteins were visualized by silver stain. The results are shown in 15 Figure 2. Markers shown in lane M on the gel represent 20 ng of Bovine serum albumin, carbonic anhydrase, B-lactoglobulin, and lysozyme in the order of descending molecular weight.

A 25 kD band appeared in fractions 25-30, a 28 kD 20 protein elutes earlier in the gradient and an 18 kD elutes later in the gradient. Figure 2 illustrates the survival activity in each of the fractions. The survival activity is noted to correspond with the presence and apparent intensity of the 25 kD protein in fractions 25- 25 30.

To demonstrate that the 25 kD band was responsible for survival promoting activity, the 25 kD protein was eluted from the polyacrylamide gel after electrophoresis and assayed for survival activity in the SCG assay. 30 After electrophoresis of 150  $\mu$ l of the SP SEPHAROSE® 1.0 M NaCl fraction in one lane of a non-reducing 14% SDS-polyacrylamide gel as above, the lane was cut into 12 slices and each slice was crushed and eluted by diffusion with rocking in buffer containing 25 mM HEPES, pH 7.4, 35 0.5 M NaCl, 0.02% Tween-20 for 18 hr at 25°C. BSA was added to the eluate to a final concentration of 200  $\mu$ g/ml

and the eluate was filtered through a 0.45 micron filter to remove acrylamide gel fragments. The filtrate was then added to a SP SEPHAROSE® column to concentrate and purify the sample. Before eluting the sample, the column  
5 was washed once in 400 µl 25 mM HEPES, pH 7.4 buffer containing 0.5 M NaCl, 0.02% Tween-20 and 200 µg BSA per ml and once in 400 µl 25 mM HEPES, pH 7.4 buffer containing 0.02% Tween-20 and 200 µg BSA per ml. The column was then washed again in 400 µl of 25 mM HEPES, pH  
10 7.4 buffer containing 0.5 M NaCl, 0.02% TWEEN 20 and 200 ug BSA per ml. The sample was eluted with 25 mM HEPES, pH 7.4 buffer containing 1.0 M NaCl, 0.02% Tween-20 and 200 µg BSA per ml. Samples were then analyzed for survival activity. Only the slice corresponding to the  
15 25 kD band showed evidence of survival activity. The 25 kD protein purified from CHO cell conditioned media is believed to be a homodimer.

The yield from the purification above was typically 1-1.5 µg from 50 liters of CHO cell conditioned medium.  
20 Overall recovery is estimated to be 10-30%, resulting in a purification of approximately 390,000 fold.

#### Example 2

This example illustrates the characterization of  
25 neurturin and several members of the TGF-β family of growth factors in the SCG assay and the lack of cross reactivity of anti-GDNF antibodies with neurturin.

The SCG assay of the purified protein indicated that the factor is maximally active at a concentration of  
30 approximately 3 ng/ml or approximately 100 pM and the EC<sub>50</sub> was approximately 1.5 ng/ml or approximately 50 pM in the expected range for a diffusible peptide growth factor (Figure 4).

Several members of the TGF-β family influence  
35 neuropeptide gene expression in sympathetic neurons, while others promote survival of different neuronal

populations. Neurturin, which is a distant member of this family of proteins, is capable of promoting virtually complete survival of sympathetic neurons for 3 days. In addition, further culturing of the SCG cells  
5 revealed that neurturin could continue to maintain these neurons for at least 10 days after withdrawal of NGF.

We tested several other members of the TGF- $\beta$  family for their ability to promote survival in the SCG assay including TGF- $\beta$ 1, activin, BMP-2, BMP-4, BMP-6 and GDNF.  
10 Of these factors, only GDNF had survival promoting activity, however, the activity of GDNF was much less potent than neurturin in this activity showing an EC<sub>50</sub> of 2-4 nM in the 3-day survival assay. The GDNF tested in this assay was rhGDNF produced in *E. Coli* obtained from  
15 Prepro Tech, Inc., Rocky Hill, N.J. The duration of action of GDNF was also less than that of neurturin inasmuch as the ability of 3DNF (50 ng/ml) to maintain survival longer than 3 day was substantially diminished. These experiments suggest the possibility that GDNF is a  
20 weak agonist for the neurturin receptor. Furthermore, the inability of activin and BMP-2 to promote survival, in contrast to their strong induction of transmitter-related gene expression in these neurons (Fann and Paterson, *Int J Dev Neurosci* 13:317-330, 1995; Fann and  
25 Patterson, *J Neurochem* 61:1349-1355, 1993) suggests that they signal through alternate receptors or signal transduction pathways.

To determine the cross-reactivity of anti-GDNF antibodies with partially purified neurturin, SCG  
30 neurons, that had been dissected and plated as described in Example 1 were treated on Day 6 with 1 ng/ml, 3 ng/ml, 10 ng/ml, or 30 ng/ml GDNF (Prepro Tech, Inc, Rocky Hill, N.J.) in the presence of anti-NGF alone, or in the presence of anti-NGF and anti-GDNF (goat IgG antibody to  
35 *E. coli*-derived rhGDNF, R & D Systems, Minneapolis, Minn). A partially purified 1.0 M SP Sepharose fraction

of neurturin was used in the assay at the approximate concentrations of 375 pg/ml, 750 pg/ml, 1.5 ng/ml and 3 ng/ml. This fraction was tested in the presence of anti-NGF alone, and in the presence of anti-NGF and  
5 anti-GDNF. The anti-GDNF antibody blocked the survival promoting activity of GDNF at a concentration up to 30 ng/ml, but did not block the survival promoting activity of neurturin.

10

## Example 3

This example illustrates the effect of neurturin on sensory neurons in a nodose ganglion survival assay.

CHO cell conditioned media that had been partially purified on the SP Sepharose column was assayed for  
15 neurotrophic activity on sensory neurons using nodose ganglia. The survival assay is a modification of that previously reported above for superior cervical ganglia. Primary dissociated cultures of nodose ganglia were prepared by dissecting tissue from E18 Sprague Dawley rat  
20 pups. The nodose ganglia were placed in Leibovitz's L15 with 2 mM l-glutamine (Cat# 11415-023, GIBCO-BRL, Gaithersburg, MD) as the tissues was dissected, digested for 30 min with 1 mg/ml collagenase (Cat#4188, Worthington Biochemical, Freehold, New Jersey) in  
25 Leibovitz's L15 medium at 37°C, followed by 30 min digestion in trypsin (lyophilized and irradiated, type TRLVMF, Cat #4454 Worthington Biochemical, Freehold, NJ), and resuspension to a final concentration of 0.25% in modified Hank's Balanced Salt Solution (Cat#H8389, Sigma  
30 Chemical Co., St. Louis, Mo). The digestion was stopped using AMO-BDNF100, a medium containing Minimum Essential Medium with Earle's salts and without l-glutamine (#11090-016 GIBCO-BRL), 10% fetal Calf Serum (Cat#1115, Hyclone Laboratories, Logan, UT), 2 mM l-glutamine  
35 (Cat#G5763 Sigma Chemical Co., St. Louis, Mo.), 20 µM FuDr (F-0503, Sigma Chemical Co.), 20 µM Uridine (Cat

#3003, Sigma Chemical Co., St. Louis, Mo.) 100 U/ml penicillin, 100 µg/ml Streptomycin, and 100 ng Brain Derived Neurotropic Factor (BDNF, Amgen, Thousand Oaks, CA). The cells were dissociated into a suspension of  
5 single cells using a silanized and flame-polished Pasteur pipet in the AMO-BDNF100 medium, and preplated on a 100 mm Falcon or Primaria culture dish (Becton Dickinson Labware, Lincoln Park, NJ) to remove non-neuronal cells. After 2 hours, the medium containing the unattached  
10 neuronal cells was removed from these dishes and triturated again through a silanized and flame-polished Pasteur pipet. The single cell suspension was plated on 24-well tissue culture plates (Costar, Wilmington, MA) that have been previously coated with a double layer of  
15 collagen, one layer of which had been ammoniated and a second layer that had been air dried. Ganglia from ten E18 rat embryos were dissociated into 2.5 mls of media and 100 µl of this suspension was added to each well. The cells were allowed to attach for 30 min in a 37°C  
20 incubator with 5% CO<sub>2</sub>/95% air. The wells were fed with AMO-BDNF100 media overnight.

The next day the cells were washed 3 times for 20 min each time with AMO medium containing no BDNF. The wells were fed with 0.5 ml of this media alone or this  
25 media containing either 50 ng/ml NGF, 100 ng/ml BDNF (Amgen, Thousand Oaks, CA), 100 ng/ml GDNF (Prepro Tech, Inc., Rocky Hill, N.J) or 3 ng/ml Neurturin. The cells were incubated at 37°C in a 5% CO<sub>2</sub>/95% air incubator for 3 days, fixed with 10% formalin, stained with crystal  
30 violet (1 µl/ml 10% formalin) and counted. Survival was ascertained as noted previously.

The neuronal Death at 72 hours is shown in Figure 10. Neuronal survival of nodose neurons cultured in BDNF has been previously reported (Thaler et al, *Develop*  
35 *Biol* 161:338-344, 1994 which is incorporated by reference). This was used as the standard for survival



for these neurons and given the value of 100% survival. Nodose ganglia that had no trophic support (AMO) showed 20%-30% survival, as did neurons that were cultured in the presence of 50 ng/ml NGF. Neurons cultured in the presence of 3 ng/ml neurturin and absence of BDNF showed survival similar to those neurons cultured in the presence of BDNF (100 ng/ml). GDNF at a concentration of 100 ng/ml promoted greater survival of nodose neurons than did BDNF (100 ng/ml). Similar findings with GDNF were recently reported for sensory neurons from chicken (Ebendal, T. et al, *J Neurosci Res* 40:276-284 1995 which is incorporated by reference).

#### Example 4

This example illustrates the determination of partial amino acid sequences of neurturin isolated from CHO cell conditioned medium.

To obtain N-terminal amino acid sequence from a purified preparation of approximately 1 µg of neurturin, the Mono S fractions 26-29 containing the peak of activity were concentrated to 25 µl by centrifuge ultrafiltration in a microcon-3 concentrators (Amicon, Inc., Beverley, MA) and loaded onto a non-reducing 14% SDS polyacrylamide gel. After electrophoretic separation, proteins were electroblotted to a PVDF membrane (Bio-Rad, Hercules, CA) and stained with 0.1% Coomassie Blue. The 25 kD band was excised and inserted into the reaction cartridge of an automated sequencer (Model 476, Applied Biosystems (Foster City, CA). Phenylthiohydantoin-amino acid (PTH-aa) recovery in the first 2-3 cycles of automated sequencing by Edman degradation indicated a sequencing yield of 4 pmoles, which was approximately 10% of the estimated amount of protein loaded on the SDS gel.

Two N-terminal sequencing runs were performed from two 50 liter purification preparations. In the first

run, 1 µg of protein in 3 pooled fractions of 1.5 ml total volume were concentrated to 25 µl and electroblotted at 100V for 2 hrs at 25°C using an electroblot buffer of 10 mM CAPS pH 11.0 buffer (Sigma, St. Louis, MO) containing 5% methanol. The amino acid sequence was obtained from 13 cycles of Edman degradation and the sequencing yield was 4 pmoles as above.

In the second run, 1.5 µg of protein in 4 pooled fractions of 2.0 ml total volume were concentrated to 25 µl and electroblotted at 36V for 12 hours at 4°C using an electroblot buffer of 25 mM Tris, 192 mM glycine, 0.04% SDS and 17% MeOH. Sequencing yield was 15 pmoles and the sequence after 16 cycles was SGARPXGLRELEVSVS (SEQ ID NO:3). The sequence obtained after 16 cycles corresponded to the shorter sequence obtained in the first run. Definite assignments could not be made at 3 of the amino acid residues in the sequence (residues 1, 6 and 11 from the N-terminal). A search of protein databases did not detect any significantly homologous sequences, suggesting that the purified factor was a novel protein.

This initial N-terminal amino acid sequence data did not enable the isolation of cDNA clones using degenerate oligonucleotides as PCR primers or probes for screening libraries. To facilitate these approaches, additional protein was purified in order to obtain internal amino acid sequence from proteolytic fragments. To obtain internal amino acid sequence from neurturin, an additional 50 liters of CHO cell conditioned medium was purified using only the first 3 chromatographic steps as outlined above, except that the gradient used to elute the Cu++ Chelating Superose column was as follows: 0-60 mM glycine (4 ml), 60mM glycine (10ml), 60-300 mM glycine (32 ml). Fractions No. 20-23 containing neurturin were concentrated to 25 µl by ultrafiltration (Amicon microcon 3, Amicon, Beverley, MA) and loaded on a non-reducing SDS

polyacrylamide gel. After electrophoresis, the gel was stained with Coomassie blue and the 25 kD neurturin band was excised. Neurturin was digested in the gel slice with endoproteinase Lys-C, and the eluted proteolytic fragments were purified by reverse phase HPLC. Only one peak was observed upon HPLC separation of the eluted peptides, which yielded amino acid sequence information for 23 cycles at the 1 pmole signal level using the automated sequencer, (internal fragment P2, SEQ ID NO:5).

10 Amino acid analysis performed on 10% of the above sample before subjecting it to digestion had indicated that 150 pmoles of protein were present in the gel slice, consisting of 7.6% lysine and 19.5% arginine. The single low level peak from the Lys-C digestion suggested that

15 the digestion and elution of peptides were inefficient. The same gel slice was redigested with trypsin and the eluted peptides separated by HPLC. Two peaks were observed on HPLC, resulting in the elucidation of two additional 10 residue amino acid sequences (4-5 pmole

20 signal level, internal fragment P1, SEQ ID NO:4 and internal fragment P3, SEQ ID NO:6) that were distinct from the N-terminal and previous internal amino acid sequences. The in situ digestion, elution and purification of peptides, and peptide sequencing was

25 performed by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University according to standard protocols for this service.

#### Example 5

30 The following example illustrates the isolation and sequence analysis of mouse and human neurturin cDNA clones.

Degenerate oligonucleotides corresponding to various stretches of confident amino acid sequence data were

35 synthesized and used as primers in the polymerase chain reaction (PCR) to amplify cDNA sequences from reverse

transcribed mRNA. A forward primer (M1676;  
5'-CCNACNGCNTAYGARGA, SEQ ID NO:50) corresponding to  
peptide sequence P2 Xaa<sub>1</sub>-Xaa<sub>2</sub>-Val-Glu-Ala-Lys-Pro-Cys-Cys-  
Gly-Pro-Thr-Ala-Tyr-Glu-Asp-Xaa<sub>3</sub>-Val-Ser-Phe-Leu-Ser-Val  
5 where Xaa<sub>1</sub> and Xaa<sub>2</sub> were unknown, Xaa<sub>3</sub> was Gln or Glu (SEQ  
ID NO:5) in combination with a reverse primer (M1677;  
5'-ARYTCYTGNAANGTRTGRTA (SEQ ID NO:52) corresponding to  
peptide sequence P3  
(Tyr-His-Thr-Leu-Gln-Glu-Leu-Ser-Ala-Arg) (SEQ ID NO:6)  
10 were used to amplify a 69 nucleotide product from cDNA  
templates derived from E21 rat and adult mouse brain.  
The PCR parameters were: 94°C for 30 sec; 55°C for 30  
sec; 72°C for 1 min for 35 cycles. The product was  
subcloned into the Bluescript KS plasmid and sequenced.  
15 All nucleotide sequencing was performed using fluorescent  
dye terminator technology per manufacturer's instructions  
on an Applied Biosystems automated sequencer Model #373  
(Applied Biosystems, Foster City, CA). Plasmid DNA for  
sequencing was prepared using the Wizard Miniprep kit  
20 (Promega Corp., Madison, WI) according to the  
manufacturer's instructions. The sequence of the  
amplified product correctly predicted amino acid sequence  
data internal to the PCR primers.

Primers corresponding to the amplified sequence were  
25 used in combination with the degenerate primers in the  
rapid amplification of cDNA ends (RACE) technique  
(Frohman, M.A. *Methods in Enzymology* 218:340-356, 1993)  
using the Marathon RACE kit (CLONTECH, Palo Alto, CA) per  
the manufacturer's instructions, except that first strand  
30 cDNA synthesis was carried out at 50°C using Superscript  
II reverse transcriptase (Gibco-BRL). Briefly, a double  
stranded adaptor oligonucleotide was ligated to the ends  
of double stranded cDNA synthesized from postnatal day 1  
rat brain mRNA. Using nested forward neuriturin PCR  
35 primers (M1676; 5'-CCNACNGCNTAYGARGA, SEQ ID NO:50 and  
1678; 5'-GACGAGGGTCCTTCCTGGACGTACACA, SEQ ID NO:53) in

combination with primers to the ligated adaptor supplied in the kit (AP1, AP2), the 3' end of the neurturin cDNA was amplified by two successive PCR reactions (1st: M1676 and AP1, using 94°C for 30 sec, 55°C for 30 sec and 72°C for 2 min for 35 cycles; 2nd: M1678 and AP2 using 94°C for 30 sec and 68°C for 2 min for 35 cycles). A 5' portion of the rat neurturin cDNA was obtained by two successive PCR reactions using the linkered cDNA as template. The 1st reaction utilized primers M1677 (SEQ ID NO:52) and AP1; using 94°C for 30 sec; 55°C for 30 sec; and 72°C for 2 min for 35 cycles. The 2nd reaction used M1679 5'-TAGCGGCTGTGTACGTCCAGGAAGGACACCTCGT (SEQ ID NO:54) and AP2 at 94°C for 30 sec and 68°C for 2 min for 35 cycles. These reactions resulted in a truncated form of the 5' end of the neurturin cDNA, apparently the result of premature termination of the cDNA during reverse transcription. The 5' and 3' RACE products were subcloned into the plasmid Bluescript KS and sequenced. The sequence of these 3' and 5' RACE products resulted in a partial rat neurturin cDNA sequence of 220 nt. Primers (#467921 5'-CAGCGACGACGCGTGCGCAAAGAGCG, SEQ ID NO:55; and M1679 (SEQ ID NO:54) corresponding to the partial rat cDNA sequence were used (PCR parameters 94°C for 30 sec and 68°C for 1 min for 35 cycles) to amplify a 101 nucleotide PCR product from mouse genomic DNA which was homologous to rat neurturin cDNA sequence.

These primers were then used to obtain murine neurturin genomic clones by amplifying gene fragments in a mouse 129/Sv library in a P1 bacteriophage vector (library screening service of Genome Systems, Inc., St. Louis, MO). A 1.6 kb Nco I fragment from this P1 clone containing the neurturin gene was identified by hybridization with primer (#465782; 5'-TAYGARGACGAGGTGTCCTTCCTGGACGTACACAGCCGCTAYCAYAC, SEQ ID NO:56). This Nco I fragment was sequenced and found to contain a stretch of coding sequence corresponding to

the N-terminal and internal amino acid sequences obtained from sequencing the active protein isolated from CHO cell conditioned media. Beginning at the N-terminal amino acid sequence of the purified protein, this nucleotide  
5 sequence encodes a 100 amino acid protein with a predicted molecular mass of 11.5 kD. A search of protein and nucleic acid databases identified neurturin as a novel protein that is approximately 40% identical to glial derived neurotrophic factor (GDNF). GDNF was  
10 purified and cloned as a factor which promotes the survival of midbrain dopaminergic neurons and is a distantly related member of the TGF- $\beta$  superfamily, which now includes more than 25 different genes that possess a wide variety of proliferative and differentiative  
15 activities. Although GDNF is less than 20% identical to any other member of the TGF- $\beta$  family, it contains the 7 cysteine residues which are conserved across the entire family and believed to be the basis of a conserved cysteine knot structure observed in the crystal structure  
20 determination of TGF- $\beta$ 2. Neurturin also contains these 7 cysteine residues, but like GDNF is less than 20% homologous to any other member of the TGF- $\beta$  family. Thus, neurturin and GDNF appear to represent a subfamily of growth factors which have significantly diverged from  
25 the rest of the TGF- $\beta$  superfamily.

To determine the sequence of the full length mouse neurturin cDNA, 5' and 3' RACE PCR was performed as above for the rat, using nested primers predicted from the mouse genomic sequence and cDNA from neonatal mouse  
30 brain. The 1st reaction for the 3' end used primers: M1777 5'-GCGGCCATCCGCATCTACGACCGGG (SEQ ID NO:57) and AP1 at 94°C for 30 sec; 65°C for 15 sec; and 68°C for 2 min for 35 cycles. The 2nd reaction used primer #467921 (SEQ ID NO:55) and AP2 at 94°C for 30 sec; 65°C for 15 sec;  
35 and 68°C for 2 min for 20 cycles. The 5' end was obtained using for the 1st reaction primer M1759,

5'-CRTAGGCCGTCGGGCGRCARCACGGGT (SEQ ID NO:58) and AP1 at 94°C for 30 sec; 65°C for 15 sec; and 68°C for 2 min for 35 cycles. The 2nd reaction used primer M1785, 5'-GCGCCGAAGGCCAGGTCGTAGATGCG (SEQ ID NO:59) and AP2 at 94°C for 30 sec; 65°C for 15 sec; and 68°C for 2 min for 20 cycles. Both sets of PCR reactions included 5% DMSO. The 5' and 3' mouse RACE products were subcloned into the plasmid Bluescript KS and sequenced. Using the sequence of RACE products, a 1.0 kb mouse neurturin cDNA sequence can be assembled. This cDNA sequence contains an open reading frame of 585 nucleotides that encodes a protein with a molecular mass of 24 kD. This full length mouse cDNA sequence is shown in Figure 7 (SEQ ID NO:12). Consistent with the processing events known to occur for TGF- $\beta$  family members, the 24 kD neurturin protein contains an amino terminal 19 amino acid signal sequence followed by a pro-domain which contains an RXXR proteolytic processing site immediately before the N-terminal amino acid sequence obtained when sequencing the protein purified from CHO cell conditioned media. Using these landmarks, the 11.5 kD mature neurturin molecule is predicted to be 11.5 kD and, by analogy to other members of the TGF- $\beta$  family, is predicted to form a disulfide linked homodimer of 23 kD, consistent with the 25 kD mass of the protein purified from CHO cell conditioned media as estimated by SDS-PAGE analysis.

For isolation of human genomic clones, primers (#467524; 5'-CGCTACTGCGCAGGCGCGTGCGARGCGGC, SEQ ID NO:60 and #10005, 5'-CGCCGACAGCTCTTGCAGCGTRTGGA, SEQ ID NO:61) predicted from the sequence of mouse neurturin were used to amplify (PCR parameters: Initial denaturation at 95°C for 1 min 30 sec followed by 94°C for 30 sec; 60°C for 15 sec; and 68°C for 60 sec for 35 cycles) a 192 nucleotide fragment from human genomic DNA. The sequence of the PCR product demonstrated that it was the human homolog of mouse neurturin. The primers were then used to screen a

human genomic library constructed in the P1 vector (library screening service, Genome Systems, Inc.) and two clones containing the human neurturin genomic locus were obtained.

- 5        The same strategy was used to determine the human sequence as discussed above for the mouse sequence. An oligo (#30152, GACCTGGGCCTGGGCTACGCGTCCGACGAG, SEQ ID NO:62) was used as a probe in a Southern blot analysis to  
10        identify restriction fragments of the P1 Clones which contained the human neurturin coding sequence. These restriction fragments (Eag I, Pvu II, Hind III, Kpn I) were subcloned into the Bluescript KS plasmid and sequenced.

- 15        The results of subcloning and sequencing of human genomic fragments were as follows. The Eag I fragment was found to be approximately 6 kb in size with the 3' Eag I site located 60 bp downstream from the stop codon. The Pvu II fragment was approximately 3.5 kb in size with the 3' Pvu II site located 250 bp downstream from the  
20        stop codon. The Hind III fragment was approximately 4.8 kb in size with the 3' Hind III site located 3kb downstream from the stop codon. The Kpn I fragment was approximately 4.2 kb in size with the 3' Kpn I site located 3.1 kb downstream from the stop codon.

- 25        The second coding exon was sequenced using these subcloned fragments. In addition, sequence was obtained from 250 bp flanking the 3' side of the second exon. The sequence was also obtained from 1000 bp flanking the 5' side of the coding exon. From these flanking sequences,  
30        forward primer 30341 (5'-CTGGCGTCCCAMCAAGGGTCTTCG-3', SEQ ID NO:71) and reverse primer 30331 (5'-GCCAGTGGTGCCGTCGAGGCGGG-3', SEQ ID NO:72) were designed so that the entire coding sequence of the second exon could be amplified by PCR.

- 35        The first coding exon was not mapped relative to the restriction sites above but was contained in the Eag I



fragment. The sequence of this exon was obtained from the subcloned Eag I fragment using the mouse primer 466215 (5'-GGCCCAGGATGAGGCGCTGGAAGG-3', SEQ ID NO:73), which contains the ATG initiation codon. Further  
5 sequence of the first coding exon was obtained with reverse primer 20215 (5'-CCACTCCACTGCCTGAWATTWCACCCC-3', SEQ ID NO:74), designed from the sequence obtained with primer 466215. Forward primer 20205 (5'-  
10 CCATGTGATTATCGACCATTCGGC-3', SEQ ID NO:75) was designed from sequence obtained with primer 20215. Primers 20205 and 20215 flank the coding sequence of the first coding exon and can be used to amplify this coding sequence using PCR.

15

## Example 6

This example illustrates the preparation of expression vectors containing neurturin cDNA.

For expression of recombinant neurturin in mammalian cells the neurturin vector pCMV-NTN-3-1 was constructed.  
20 The 585 nucleotide open reading frame of the neurturin cDNA was amplified by PCR using a primer containing the first 27 nucleotides of the neurturin coding sequence (5'-GCGACGCGTACCATGAGGCGCTGGAAGGCAGCGGCCCTG, SEQ ID NO:63) and a primer containing the last 5 codons and the  
25 stop codon (5'-GACGGATCCGCATCACACGCACGCGCACTC) (SEQ ID NO:64) using reverse transcribed postnatal day 1 mouse brain mRNA as template using (PCR parameters: 94°C for 30 sec; 60°C for 15 sec; and 68°C for 2 min for 35 cycles and including 5% DMSO in the reaction). The PCR product  
30 was subcloned into the Eco RV site of BSKS and sequenced to verify that it contained no PCR generated mutations. The neurturin coding sequence was then excised from this vector using Mlu I (5' end) and Bam HI (3' end) and inserted downstream of the CMV IE promoter/enhancer in  
35 the mammalian expression vector pCB6 (Brewer, C.B.

*Methods in Cell Biology* 43:233-245, 1994) to produce the pCMV-NTN-3-1 vector using these sites.

For expression of recombinant protein in *E. Coli*, the mature coding region of mouse neurturin was amplified by PCR using a primer containing the first 7 codons of the mature coding sequence (5'-GACCATATGCCGGGGGCTCGGCCTTGTGG) (SEQ ID NO:65) and a primer containing the last 5 codons and the stop codon 5'-GACGGATCCGCATCACACGCACGCGCACTC (SEQ ID NO:66) using a fragment containing the murine neurturin gene as template using (PCR parameters: 94°C for 30 sec; 60°C for 15 sec and 68°C for 90 sec for 25 cycles with 5% DMSO added into the reaction). The amplified product was subcloned into the Eco RV site of BSKS, the nucleotide sequence was verified, and this fragment was then transferred to the expression vector pET-30a (Novagen, Madison, WI) using an Nde I site (5' end) and an Eco RI site (3' end). The pET-neurturin (pET-NTN) vector codes for an initiator methionine in front of the first amino acid of the mature mouse neurturin protein predicted from the N-terminal amino acid sequence of neurturin purified from the CHO cell conditioned media.

#### Example 7

This example illustrates the transient transfection of NIH3T3 cells with the neurturin expression vector pCMV-NTN-3-1 and that the product of the genomic sequence in Example 5 is biologically active.

To demonstrate that the cloned neurturin cDNA was sufficient to direct the synthesis of biologically active neurturin we transiently introduced the pCMV-NTN-3-1 plasmid into NIH3T3 cells using the lipofectamine method of transfection. NIH3T3 cells were plated at a density of 400,000 cells per well (34.6 mm diameter) in 6 well plates (Corning, Corning, NY) 24 hours before transfection. DNA liposome complexes were prepared and

added to the cells according to the manufacturer's protocol using 1.5 µg CMV-neurturin plasmid DNA (isolated and purified using a Qiagen (Chatsworth, CA) tip-500 column according to manufacturer's protocol) and 10 µl lipofectamine reagent (Gibco BRL, Gaithersburg, MD) in 1:1 DME/F12 medium containing 5 µg/ml insulin, 5 µg/ml transferrin, and 5 ng/ml sodium selenite (Sigma, St. Louis, MO). Five hours after the addition of DNA liposome complexes in 1 ml medium per well, 1 ml DME medium containing 20% calf serum was added to each well. Twenty-four hours after the addition of DNA-liposome complexes, the 2 ml medium above was replaced with 1 ml DME medium containing 10% calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 µ/ml streptomycin, and 25 µg/ml heparin. The cells were incubated for an additional 24 hours before the conditioned medium was harvested, centrifuged to remove cellular debris, and frozen.

As a control, NIH3T3 cells were transfected as above using 1.5 µg CMV-neo expression plasmid (containing no cDNA insert) in place of the 1.5 µg CMV-neurturin plasmid. Conditioned medium from NIH3T3 cells transfected with either control plasmid or CMV-neurturin plasmid was assayed by direct addition to the SCG culture medium at the time of NGF deprivation. Addition of 0.25 ml conditioned medium from CMV-neurturin-transfected cells promoted 70% survival of sympathetic neurons, and >90% survival could be obtained with 0.45 ml of this conditioned medium. No significant survival promoting activity was detected in the conditioned medium of control transfected NIH3T3 cells.

#### Example 8

This example illustrates the preparation of Chinese hamster ovary cells stably transformed with neurturin cDNA.

DG44 cells, a Chinese hamster ovary cell derivative that is deficient in dihydrofolate reductase (DHFR) (Urlaub et al *Cell* 3:405-412, 1983 which is incorporated by reference), were stably co-transfected with expression plasmid (pCMV-NTN-3-1) and a DHFR expression plasmid (HLD) (McArthur, and Stanners *J. Biol. Chem.* 266:6000-6005, 1991 which is incorporated by reference).

On day 1 DG44 cells were plated at  $1 \times 10^6$  cells per 10 cm plate in Ham's F12 medium with 10% fetal calf serum (FCS). This density must not be exceeded or cells will overgrow before selection media is added on day 5.

On day 2 cells were transfected with a 9:1 ratio of pCMV-NTN to DHFR expression plasmid using the calcium phosphate method (10 ug DNA /10 cm plate) (Chen and Okayama, *Mol Cell Biol* 7:2745-2752, 1987 which is incorporated by reference).

On day 3 the transfected cells were washed with Ham's F12 medium and fed Ham's F12 with 10% FCS.

On day 5 the cells were washed with MEM alpha medium and fed selection medium, which is MEM alpha with 10% FCS and 400 ug/ml G418. The cells were maintained in selection media, feeding every 4 days. Colonies began to appear approximately 14 days after transfection. Colonies growing in selection media were then transferred to a 24 well plate and trypsinized the next day to disperse the cells. The cells were grown to confluence in either 24 well or 6 well plates in order to screen the cells for expression of recombinant protein. Expression of neurturin was examined in 10 clonal lines and two high expressing lines were detected using the SCG survival assay. These clonal lines were expanded and expression in these selected cell lines was amplified by selection in 50 nM methotrexate (MTX). For selection in MTX, cells were grown to 50% confluence in a 150 cm<sup>2</sup> flask in selection medium. The medium was changed to MEM alpha containing 50 nM MTX concentration (it was not necessary

to use G418 during MTX amplification). After placement in 50 nM MTX, the majority of cells died and colonies of resistant cells reappeared in 1-2 weeks. At this time, the cells were trypsinized to disperse colonies and are  
5 split when cells reach confluence. Cells eventually reached the same growth rate as before. The selected cells were screened for expression of recombinant protein. A 2-3 fold increase in expression was observed after selection in 50 nM MTX. Frozen stocks were kept  
10 for cell lines obtained from the original selection and the 50 nM MTX selection. Further selection could be continued in increasing MTX until desired levels of expression are obtained.

Using the above method, we isolated cells identified  
15 as DG44CHO5-3(G418)(pCMV-NTN-3-1) and DG44CHO5-3(50nMTX)(pCMV-NTN-3-1). Cells from the DG44CHO5-3(50nMTX)(pCMV-NTN-3-1) strain expressed levels of approximately 100 µg of biologically active protein per liter of conditioned media determined by direct assay of  
20 conditioned medium in SCG assay according to the methods in example 1.

#### Example 9

This example illustrates the expression of neurturin  
25 in various tissues.

A survey of neurturin and GDNF expression was performed in rat embryonic tissues (E10, day 10 after conception), neonatal tissues (P1, Postnatal Day 1), and adult tissues (> 3 mos) using semi-quantitative RT/PCR  
30 (Estus et al., *J Cell Biol* 127:1717-1727, 1994 which is incorporated by reference). The RNA samples were obtained from various tissues and PCR products were detected either by autoradiography after incorporation of  $\alpha$ -<sup>32</sup>P-dCTP in the PCR and electrophoresis on a  
35 polyacrylamide gel (Figure 6) or by ethidium bromide staining of DNA after electrophoresis on agarose gels

(Tables 3 and 4). The neurturin fragment of 101 base pairs was obtained using the forward primer CAGCGACGACGCGTGCGCAAAGAGCG (SEQ ID NO:67) and reverse primer TAGCGGCTGTGTACGTCCAGGAAGGACACCTCGT (SEQ ID NO:68) and the GDNF fragment of 194 base pairs was obtained using the forward primer AAAAATCGGGGGTGYGTCTTA (SEQ ID NO:69) and the reverse primer CATGCCTGGCCTACYTTGTCA (SEQ ID NO:70).

No neurturin or GDNF mRNA was detected at the earliest embryonic age (embryonic day 10, E10) surveyed.

In neonates (postnatal day 1, P1) both transcripts were expressed in many tissues although neurturin tended to show a greater expression in most tissues than did GDNF. (see table 3).

Table 3.

15

	NEURTURIN	GDNF
Liver	+++	-
Blood	+++	+
20 Thymus	+	-
Brain	++	+
Sciatic nerve	-	+
Kidney	++	++
25 Spleen	++	+
Cerebellum	++	+
Heart	++	+
Bone	+	+

30 As shown in Table 3, differences in the tissue distributions of neurturin and GDNF were noted. In particular, no GDNF was detected in liver and thymus where neurturin expression was detected and no neurturin was detected in sciatic nerve where GDNF was detected.

35 Neurturin and GDNF mRNA were detected in many tissues in the adult animal, but the tissue-specific

pattern of expression for these two genes was very different. (table 4, Figure 5).

Table 4.

		NEURTURIN	GDNF
5			
	Liver	-	-
	Blood	+	-
	Thymus	+	++
	Brain	+	-
10	Sciatic nerve	-	-
	Kidney	++	+
	Spleen	-	+
	Cerebellum	-	-
15	Uterus	++	-
	Bone marrow	++	-
	Testis	++	++
	Ovary	+	+
	Placenta	+	-
20	Skeletal muscle	+	-
	Spinal cord	+	-
	Adrenal gland	++	++
25	Gut	+	++

As shown in table 4, neurturin was found to be expressed in brain and spinal cord as well as in blood and bone marrow where no GDNF was detected. The level of expression of neurturin in brain and blood was, however, less than that detected in neonatal tissue.

Neurturin was also highly expressed in freshly isolated rat peritoneal mast cells, whereas GDNF showed little or no expression.

This example illustrates the preparation of antisera to neurturin by immunization of rabbits with a neurturin peptide.

The peptide sequence corresponding to amino acids 5 73-87 of the mature murine neurturin protein was synthesized and coupled to keyhole limpet hemocyanin (KLH) as described earlier (Harlow and Lane, *Antibodies: a laboratory manual*, 1988. Cold Spring Harbor Laboratory, New York, NY. p. 72-81 which is incorporated by 10 reference). The KLH-coupled peptide was submitted to Caltag, Inc. and each of two rabbits were immunized. Immunization was by subcutaneous injection at 7-10 sites. The first injection was with 150 µg KLH-coupled peptide which was resuspended in 0.5 ml saline and emulsified 15 with 0.5 ml complete Freund's adjuvant. Boost injections were begun 4 weeks after the initial injection and were performed once every 7 days as above for a total of 5 injections except that 100 µg of KLH-coupled peptide and incomplete Freund's adjuvant were used. Serum samples 20 were collected 1 week after the fifth boost.

A pooled volume of twenty ml of serum that had been collected from both rabbits one week after the 5th injection was purified. For purification, a peptide affinity column was prepared by coupling the above 25 peptide to cyanogen bromide activated Sepharose 4B according to the manufacturers protocol (Pharmacia Biotech). The serum was diluted 10 fold in 10 mM Tris pH 7.5 buffer and mixed by gentle rocking for 16 hours at 4°C with 0.5 ml of peptide agarose matrix containing 5 mg 30 of coupled peptide. The matrix was placed into a column, washed with 5 ml of 10 mM Tris pH 7.5, 150 mM NaCl, washed with 5 ml of 10 mM Tris pH 7.5 buffer containing 0.4 M NaCl and eluted with 5.5 ml of 100 mM glycine pH 2.5 buffer. One tenth volume of 1.0M Tris pH 8.0 buffer 35 was added to the eluate immediately after elution to



neutralize the pH. The glycine eluate was dialyzed overnight against 10 mM Tris pH 7.5, 150 mM NaCl.

The affinity-purified antibodies were used in a western blot to demonstrate specific recognition of recombinant neurturin protein. Ten ml of conditioned medium collected from DG44CHO5-3(G418)(pCMV-NTN-3-1) cells was purified over SP Sepharose as described in Example 1 and the proteins electrophoresed on a reducing SDS-PAGE gel in the tricine buffer system (Schagger and von Jagow *Analytical Biochemistry* 166:368-379, 1987). The proteins were electroblotted to a nitrocellulose membrane in 25 mM Tris, 192 mM glycine, 0.04% SDS, 17% methanol at 4°C for 16 hr. The membrane was incubated with the affinity-purified anti-neurturin peptide antibodies and then with horseradish peroxidase-coupled sheep anti-rabbit IgG (Harlow and Lane, *supra*, p. 498-510). Bound antibodies were detected with enhanced chemiluminescence (ECL kit, Amersham, Buckinghamshire, England). The anti-neurturin antibodies recognized a single, approximately 11.5 kD protein band in the conditioned medium of the DG44CHO5-3(G418)(pCMV-NTN-3-1) cells. Using these anti-neurturin antibodies, neurturin protein could be detected in 10 ml of conditioned medium from DG44CHO5-3(G418)(pCMV-NTN-3-1) cells but could not be detected in 10 ml of medium conditioned with DG44 cells that had not been transformed with the neurturin expression vector.

#### Example 11

The following example illustrates the identification of additional members of the GDNF/neurturin/persephin gene subfamily.

The TGF- $\beta$  superfamily currently contains over 25 different gene members (for review see Kingsley, *Genes and Development* 8: 133-146, 1994 which is incorporated by reference). The individual family members display

varying degrees of homology with each other and several subgroups within the superfamily can be defined by phylogenetic analysis using the Clustal V program (Higgins et al, *Comput Appl Biosci* 8: 189-191, 1992 which is incorporated by reference) and by bootstrap analysis of phylogenetic trees (Felsenstein, *Evolution* 39:783-791, 1985 which is incorporated by reference). Neurturin or persephin is approximately 40% identical to GDNF but less than 20% identical to any other member of the TGF- $\beta$  superfamily. Several sequence regions in neurturin can be identified (Figure 5) that are highly conserved within the GDNF/neurturin/persephin subfamily but not within the TGF- $\beta$  superfamily. These conserved regions are likely to characterize a subfamily containing previously unisolated genes, which can now be isolated using the conserved sequence regions identified by the discovery and sequencing of the neurturin and persephin genes. Regions of high sequence conservation between neurturin, persephin and GDNF allow the design of degenerate oligonucleotides which can be used either as probes or primers. Conserved-region amino acid sequences have been identified herein to include Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly-Tyr where Xaa<sub>1</sub> is Ser, Thr or Ala and Xaa<sub>2</sub> is Glu or Asp (SEQ ID NO:108); Glu-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Arg-Tyr-Cys-Xaa<sub>4</sub>-Gly-Xaa<sub>5</sub>-Cys in which Xaa<sub>1</sub> is Thr, Glu or lys, Xaa<sub>2</sub> is Val, Leu or Ile, Xaa<sub>3</sub> is Leu or Ile, Xaa<sub>4</sub> is Ala or Ser, and Xaa<sub>5</sub> is Ala or Ser, (SEQ ID NO:113); and Cys-Cys-Xaa<sub>1</sub>-Pro-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Xaa<sub>5</sub>-Asp-Xaa<sub>6</sub>-Xaa<sub>7</sub>-Xaa<sub>8</sub>-Phe-Leu-Asp-Xaa<sub>9</sub> in which Xaa<sub>1</sub> is Arg or Gln, Xaa<sub>2</sub> is Thr or Val or Ile, Xaa<sub>3</sub> is Ala or Ser, Xaa<sub>4</sub> is Tyr or Phe, Xaa<sub>5</sub> is Glu, Asp or Ala, Xaa<sub>6</sub> is Glu, Asp or no amino acid, Xaa<sub>7</sub> is val or leu, Xaa<sub>8</sub> is Ser or Thr, and Xaa<sub>9</sub> is Asp or Val (SEQ ID NO:114). Nucleotide sequences containing a coding sequence for the above conserved sequences or fragments of the above conserved sequences can be used as probes.

Exemplary probe and primer sequences which can be designed from these regions are as follows.

Forward primers,

5      Primer A (M3119): 5'-GTNDGNGANYTGGGNYTGGGNTA (SEQ ID NO:115) 23 nt which codes for the amino acid sequence, Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly-Tyr where Xaa<sub>1</sub> is Thr, Ser or Ala and Xaa<sub>2</sub> is Glu or Asp (SEQ ID NO:125);

10      Primer B (M3123): 5'-GANBTNWCNTTTYTNGANG (SEQ ID NO:116) 19 nt which codes for the amino acid sequence, Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Leu-Xaa<sub>4</sub>-Xaa<sub>5</sub> where Xaa<sub>1</sub> is Asp or Glu, Xaa<sub>2</sub> is Val or Leu, Xaa<sub>3</sub> is Thr or Ser, Xaa<sub>4</sub> is Asp or Glu, and Xaa<sub>5</sub> is Asp or Val (SEQ ID NO:126);

20      Primer C (M3126): 5'-GANBTNWCNTTTYTNGANGW (SEQ ID NO:117) 20 nt which codes for the amino acid sequence, Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Leu-Xaa<sub>4</sub>-Xaa<sub>5</sub> where Xaa<sub>1</sub> is Asp or Glu, Xaa<sub>2</sub> is Val or Leu, Xaa<sub>3</sub> is Thr or Ser, Xaa<sub>4</sub> is Asp or Glu, and Xaa<sub>5</sub> is Asp or Val (SEQ ID NO:126);

25      Primer D (M3121): 5'-TTYMGNTAYTGYDSNGGNDSTG (SEQ ID NO:118) 23 nt which codes for the amino acid sequence, Phe-Arg-Tyr-Cys-Xaa<sub>1</sub>-Gly-Xaa<sub>2</sub>-Cys where Xaa<sub>1</sub> is Ser or Ala and Xaa<sub>2</sub> is Ser or Ala (SEQ ID NO:127);

30      Primer E (M3122): 5'-GTNDGNGANYTGGGNYTNGG (SEQ ID NO:119) 20 nt which codes for the amino acid sequence, Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly where Xaa<sub>1</sub> is Thr, Ser or Ala and Xaa<sub>2</sub> is Asp or Glu (SEQ ID NO:128); and

35

Primer F (M3176): 5'-GTNDGNGANYTGGGNYTGGGNTT (SEQ ID NO:120) 23 nt which codes for the amino acid sequence, Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly-Phe where Xaa<sub>1</sub> is Thr, Ser or Ala and Xaa<sub>2</sub> is Glu or Asp (SEQ ID NO:129).

Reverse primers,

Primer G (M3125): 5'-WCNTCNARRAANGWNAVNTC (SEQ ID NO:121) 20 nt whose reverse complementary sequence codes for the amino acid sequence, Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Leu-Xaa<sub>4</sub>-Xaa<sub>5</sub> where Xaa<sub>1</sub> is Asp or Glu, Xaa<sub>2</sub> is Val or Leu, Xaa<sub>3</sub> is Thr or Ser, Xaa<sub>4</sub> is Asp or Glu, and Xaa<sub>5</sub> is Asp or Val (SEQ ID NO:126);

Primer H (M3124): 5'-WCNTCNARRAANGWNAVNT (SEQ ID NO:122) 19 nt whose reverse complementary sequence codes for the amino acid sequence, Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Leu-Xaa<sub>4</sub>-Xaa<sub>5</sub> where Xaa<sub>1</sub> is Asp or Glu, Xaa<sub>2</sub> is Val or Leu, Xaa<sub>3</sub> is Thr or Ser, Xaa<sub>4</sub> is Asp or Glu, and Xaa<sub>5</sub> is Asp or Val (SEQ ID NO:126);

Primer I (M3120): 5'-CANSHNCCNSHRCARTANCKRAA (SEQ ID NO:123) 23 nt whose reverse complementary sequence codes for the amino acid sequence, Phe-Arg-Tyr-Cys-Xaa<sub>1</sub>-Gly-Xaa<sub>2</sub>-Cys where Xaa<sub>1</sub> is Ser or Ala and Xaa<sub>2</sub> is Ser or Ala (SEQ ID NO:127); and

Primer J (M3118): 5'-CANSHNCCNSHRCARTANCKRAANA (SEQ ID NO:124) 25 nt whose reverse complementary sequence codes for the amino acid sequence, Xaa<sub>1</sub>-Phe-Arg-Tyr-Cys-Xaa<sub>2</sub>-Gly-Xaa<sub>3</sub>-Cys where Xaa<sub>1</sub> is Ile or Leu, Xaa<sub>2</sub> is Ser or Ala and Xaa<sub>3</sub> is Ser or Ala (SEQ ID NO:130).

In addition to the above, the following primers are based upon conserved regions in GDNF and neurturin (SEQ ID NOS:33-35).

5 Primer 1, GTNWSNGANYTNGGNYTNGGNTA (SEQ ID NO:42) which encodes the amino acid sequence, Val-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Leu-Gly-Leu-Gly-Tyr where Xaa<sub>1</sub> is Ser or Thr and Xaa<sub>2</sub> is Glu or Asp (SEQ ID NO:33);

10 Primer 2, TTYMGNTAYTGYDSNGGNDSENTGYGANKCNGC (SEQ ID NO:43) which encodes amino acid sequence Phe-Arg-Tyr-Cys-Xaa<sub>1</sub>-Gly-Xaa<sub>2</sub>-Cys-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Ala where Xaa<sub>1</sub> is Ala or Ser, Xaa<sub>2</sub> is Ala or Ser, Xaa<sub>3</sub> is Glu or Asp and Xaa<sub>4</sub> is Ser or Ala (SEQ ID NO:36);

15 Primer 3 reverse GCNGMNTCRCANSHNCCNSHRTANCKRAA (SEQ ID NO:44) whose reverse complementary sequence encodes amino acid sequence Phe-Arg-Tyr-Cys-Xaa<sub>1</sub>-Gly-Xaa<sub>2</sub>-Cys-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Ala where Xaa<sub>1</sub> is Ala or Ser, Xaa<sub>2</sub> is Ala or Ser, Xaa<sub>3</sub> is Glu or Asp and Xaa<sub>4</sub> is Ser or Ala (SEQ ID NO:37);

20

25 Primer 4 reverse TCRTCNTCRWANGCNRYNGGNCCKARCA (SEQ ID NO:45) whose reverse complementary sequence encodes amino acid sequence Cys-Cys-Arg-Pro-Xaa<sub>1</sub>-Ala-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Asp-Xaa<sub>4</sub> where Xaa<sub>1</sub> is Ile or Thr or Val, Xaa<sub>2</sub> Try or Phe, Xaa<sub>3</sub> is Glu or Asp and Xaa<sub>4</sub> is Glu or Asp (SEQ ID NO:38);

30 Primer 5 reverse TCNARRAANSWNAVNTCRTCNTCRWANGC (SEQ ID NO:46) whose reverse complementary sequence encodes amino acid sequence Ala-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Asp-Xaa<sub>3</sub>-Xaa<sub>4</sub>-Ser-Phe-Leu-Asp where Xaa<sub>1</sub> is Tyr or Phe, Xaa<sub>2</sub> Glu or Asp, Xaa<sub>3</sub> is Glu or Asp, and Xaa<sub>4</sub> is Val or Leu (SEQ ID NO:39);

Primer 6 GARRMNBNTNHTNTTYMGNTAYTG (SEQ ID NO:47)  
which encodes amino acid sequence Glu-Xaa<sub>1</sub>-Xaa<sub>2</sub>-  
Xaa<sub>3</sub>-Phe-Arg-Tyr-Cys where Xaa<sub>1</sub> is Glu or Thr, Xaa<sub>2</sub>  
is Leu or Val and Xaa<sub>3</sub> is Ile or Leu (SEQ ID  
NO:40);

5

Primer 7 GARRMNBNTNHTNTTYMGNTAYTGYDSNGGNDSTGHGA  
(SEQ ID NO:48) which encodes amino acid sequence  
Glu-Xaa<sub>1</sub>-Xaa<sub>2</sub>-Xaa<sub>3</sub>-Phe-Arg-Tyr-Cys-Xaa<sub>4</sub>-Gly-Xaa<sub>5</sub>-  
Cys-Xaa<sub>6</sub> where Xaa<sub>1</sub> is Glu or Thr, Xaa<sub>2</sub> is Leu or  
Val, Xaa<sub>3</sub> is Ile or Leu, Xaa<sub>4</sub> is Ser or Ala, Xaa<sub>5</sub>  
is Ser or Ala and Xaa<sub>6</sub> is Glu or Asp (SEQ ID  
NO:41).

10

The above sequences can be used as probes for  
screening libraries of genomic clones or as primers for  
amplifying gene fragments from genomic DNA or libraries  
of genomic clones or from reverse transcribed cDNA using  
RNA templates from a variety of tissues. Genomic DNA or  
libraries of genomic clones can be used as templates  
because the neurturin, persephin and GDNF coding  
sequences for the mature proteins are not interrupted by  
introns.

A degenerate oligonucleotide can be synthesized as  
a mixture of oligonucleotides containing all of the  
possible nucleotide sequences which code for the  
conserved amino acid sequence. To reduce the number of  
different oligonucleotides in a degenerate mix, an  
inosine or universal base (Loakes et al, *Nucleic Acids*  
*Res* 22:4039-43, 1994) can be incorporated in the  
synthesis at positions where all four nucleotides are  
possible. The inosine or universal base forms base pairs  
with each of the four normal DNA bases which are less  
stabilizing than AT and GC base pairs but which are also  
less destabilizing than mismatches between the normal  
bases (i.e. AG, AC, TG, TC).

35

To isolate family members a primer above can be end labeled with  $^{32}\text{P}$  using T4 polynucleotide kinase and hybridized to libraries of human genomic clones according to standard procedures.

- 5 A preferred method for isolating family member genes would be to use various combinations of the degenerate primers above as primers in the polymerase chain reaction using genomic DNA as a template. The various combinations of primers can include sequential
- 10 PCR reactions utilizing nested primers or the use of a forward primer paired with an oligo dT primer. In addition, one of the degenerate primers can be used with a vector primer, a single primer can be used in an inverted PCR assay or PCR can be performed with one
- 15 degenerate primer and a random primer. As an example using the above set of primers, primer 2 (SEQ ID NO:43) can be used with primer 4 (SEQ ID NO:45) in PCR with 1 ug of human genomic DNA and cycling parameters of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 60 sec. The above
- 20 PCR conditions are exemplary only and one skilled in the art will readily appreciate that a range of suitable conditions and primer combinations could be used or optimized such as different temperatures and varying salt concentrations in the buffer medium and the like. It is
- 25 preferred that DMSO be added to the PCR reaction to a final concentration of 5% inasmuch as this was found to be necessary for amplification of this region of the neurturin gene. The PCR reaction, when run on an agarose gel, should contain products in the size range of 100-150
- 30 base pairs since a one amino acid gap is introduced in the neurturin sequence and a five amino acid gap is introduced in the persephin sequence when either sequence is aligned with GDNF, and thus family member genes might also contain a slightly variable spacing between the
- 35 conserved sequences of primers 2 and 4. The PCR products in the range of 100-150 base pairs should contain

multiple amplified gene products including GDNF, neurturin and persephin as well as previously unisolated family members. To identify sequences of these products, they can be gel purified and ligated into the Bluescript  
5 plasmid (Stratagene), and then transformed into the XL1-blue E. Coli host strain (Stratagene). Bacterial colonies containing individual subclones can be picked for isolation and plated on nitrocellulose filters in two replicas. Each of the replicate filters can be screened  
10 with an oligonucleotide probe for either unique GDNF or unique neurturin or unique persephin sequence in the amplified region. Subclones not hybridizing to either GDNF or neurturin or persephin can be sequenced and if found to encode previously unisolated family members, the  
15 sequence can be used to isolate full length cDNA clones and genomic clones as was done for neurturin (Example 5). A similar method was used to isolate new gene members (GDF-3 and GDF-9) of the TGF- $\beta$  superfamily based on homology between previously identified genes (McPherron *J*  
20 *Biol Chem* 268: 3444-3449, 1993 which is incorporated by reference).

The inventors herein believe that the most preferred way to isolate family member genes may be to apply the above PCR procedure as a screening method to  
25 isolate individual family member genomic clones from a library. This is because there is only one exon for the coding region of both mature neurturin and GDNF. If, for example, the above PCR reaction with primers 2 and 4 generates products of the appropriate size using human  
30 genomic DNA as template, the same reaction can be performed using, as template, pools of genomic clones in the P1 vector according to methods well known in the art, for example that used for isolating neurturin human  
35 genomic clones (Example 5). Pools containing the neurturin gene in this library have previously been identified and persephin and GDNF-containing pools can be



readily identified by screening with GDNF specific primers. Thus non-neurturin, non-persephin, non-GDNF pools which generate a product of the correct size using the degenerate primers will be readily recognized as previously unisolated family members. The PCR products generated from these pools can be sequenced directly using the automated sequencer and genomic clones can be isolated by further subdivision and screening of the pooled clones as a standard service offered by Genome Systems, Inc.

#### Example 12

The following example illustrates the isolation and identification of persephin utilizing the procedures and primers described in Example 11.

The degenerate PCR strategy devised by the inventors herein has now been successfully utilized to identify a third factor, persephin, that is approximately 35-50% identical to both GDNF and neurturin. The experimental approach was described above and is provided in greater detail as follows. Primers corresponding to the amino acid sequence Val-Xaa1-Xaa2-Leu-Gly-Leu-Gly-Tyr where Xaa1 is Ser or Thr and Xaa2 is Glu or Asp (SEQ ID NO:33) [M1996; 5'-GTNWSNGANYTNGGNYTNGGNTA (SEQ ID NO:42)] and Phe-Arg-Tyr-Cys-Xaa1-Gly-Xaa2-Cys-Xaa3-Xaa4-Ala where Xaa1 is Ala or Ser, Xaa2 is Ala or Ser, Xaa3 is Glu or Asp and Xaa4 is Ser or Ala (SEQ ID NO:37) [M1999; 5'-GCNGMNTCRCANSHNCCNSHRCARTANCKRAA (SEQ ID NO:44)] were used to amplify a 77 nt fragment from rat genomic DNA using KlenTag enzyme and buffer under the following conditions: 94°C for 30 sec; 44°C for 30 sec; 72°C for 30 sec for 40 cycles. The resulting product was subcloned into the Bluescript KS plasmid and sequenced. All nucleotide sequencing was performed using fluorescent dye terminator technology per manufacturer's instructions on an Applied Biosystems automated sequencer Model #373

(Applied Biosystems, Foster City, CA). Plasmid DNA for sequencing was prepared using the Wizard Miniprep kit (Promega Corp., Madison, WI) according to the manufacturer's instructions.

- 5           The sequence of one of the amplified products predicted amino acid sequence data internal to the PCR primers that was different from that of GDNF or neurturin but had more than 20% identity with GDNF and neurturin, whereas the sequences of others we obtained corresponded  
10 to GDNF or neurturin, as would be expected. The novel sequence was thought to identify a new member of this family which we named persephin.

- The sequence of this fragment internal to the primers was 5'-TGCCTCAGAGGAGAAGATTATC (SEQ ID NO:90).  
15 This encodes the last nucleotide of the Tyr codon, and then encodes the amino acids: Ala-Ser-Glu-Glu-Lys-Ile-Ile (SEQ ID NO:91). This sequence was then aligned with the rat sequences of GDNF and neurturin. This analysis confirmed that persephin was unique.  
20 LGLGYETKEELIFRYC GDNF (rat) (SEQ ID NO:92)  
LGLGYTSDETVLFYRC NTN (rat) (SEQ ID NO:93)  
LGLGYASEEKIIFRYC PSP (rat) (SEQ ID NO:94)

- 25           To obtain additional persephin sequence, primers containing portions of the unique 22 nt of the amplified fragment above were used in the rapid amplification of cDNA ends (RACE) technique (Frohman, M.A. Methods in Enzymology 218:340-356, 1993) using the Marathon RACE kit  
30 (CLONTECH, Palo Alto, CA) per the manufacturer's instructions, except that first strand cDNA synthesis was carried out at 50°C using Superscript II reverse transcriptase (Gibco-BRL). Briefly, a double stranded adaptor oligonucleotide was ligated to the ends of double  
35 stranded cDNA synthesized from postnatal day 1 rat brain mRNA. Using nested forward persephin PCR primers, (10135; 5'-AGTCGGGGTTGGGGTATGCCTCA, SEQ ID NO:95 and M2026; 5'-TATGCCTCAGAGGAGAAGATTATCTT SEQ ID NO:96) in

combination with primers to the ligated adaptor supplied in the kit (AP1, AP2), the 3' end of the persephin cDNA was amplified by two successive PCR reactions (1st: 10135 and AP1, using 94°C for 30 sec, 60°C for 15 sec and 68°C for 2 min for 35 cycles; 2nd: M2026 and AP2 using 94°C for 30 sec, 60 for 15 sec and 68°C for 2 min for 21 cycles). An approximately 350 nt fragment was obtained from this PCR reaction and this fragment was directly sequenced using primer M2026. The sequence of this 3' RACE product resulted in a partial rat persephin cDNA sequence of approximately 350 nt (SEQ ID NO:97). The predicted amino acid sequence of this cDNA was compared to that of GDNF and neurturin, and found to be approximately 40% homologous to each of these proteins. Importantly, the characteristic spacing of the cyteine residues in members of the TGF- $\beta$  superfamily was present. Furthermore, in addition to the region of similarity encoded by the degenerate primers used to isolate persephin, another region of high homology shared between GDNF and neurturin, but absent in other members of the TGF- $\beta$  superfamily, was also present in persephin

	GDNF	ACCRPVAFDDDLFLDD	(aa 60-76) (SEQ ID NO:98)
	NTN	PCCRPTAYEDEVSKDV	(aa 61-77) (SEQ ID NO:99)
25	PSP	PCCQPTSYAD-VTFLDD	(aa 57-72) (SEQ ID NO:100)

(Amino acid numbering uses the first Cys residue as amino acid 1).

30 With the confirmation that persephin was indeed a new member of the GDNF/neurturin subfamily, we isolated murine genomic clones of persephin to obtain additional sequence information. Primers (forward, M2026; 5'-TATGCCTCAGAGGAGAAGATTATCTT, SEQ ID NO:96 and reverse, 35 M3028; 5'-TCATCAAGGAAGGTCACATCAGCATA, SEQ ID NO:101) corresponding to rat cDNA sequence were used in a PCR reaction (PCR parameters: 94°C for 30 sec, 55°C for 15 sec and 72°C for 30 sec for 35 cycles) to amplify a 155 nt fragment from mouse genomic DNA which was homologous

to rat persephin cDNA sequence. These primers were then used to obtain murine persephin genomic clones from a mouse 129/Sv library in a P1 bacteriophage vector (library screening service of Genome Systems, Inc., St.

5 Louis, MO).

Restriction fragments (3.4 kb Nco I and a 3.3 kb Bam H1) from this P1 clone containing the persephin gene were identified by hybridization with a 210 nt fragment obtained by PCR using mouse genomic DNA with primers  
10 (forward, M2026; SEQ ID NO:96 and reverse, M3159; 5'-CCACCACAGCCACAAGCTGCGGSTGAGAGCTG, SEQ ID NO:102) and PCR parameters: 94°C for 30 sec, 55°C for 15 sec and 72°C for 30 sec for 35 cycles. The Nco I and Bam H1 fragments were sequenced and found to encode a stretch of amino  
15 acids corresponding to that present in the rat persephin RACE product, as well as being homologous to the mature regions of both neurturin and GDNF (Figure 11).

When the amino acid sequences of murine GDNF, neurturin and persephin are aligned using the first  
20 cysteine as the starting point (which is done because alterations in the cleavage sites between family members creates variability in the segments upstream of the first cysteine), persephin (91 amino acids) is somewhat smaller than either neurturin (95 amino acids) or GDNF (94 amino  
25 acids). The overall identity within this region is about 50% with neurturin and about 40% with GDNF (Figure 12).

Further nucleotide sequencing of the murine persephin Nco I fragment revealed the nucleotide sequence of the entire murine persephin gene (SEQ ID NO:131;  
30 Figure 17). An open reading frame extends from the sequence coding for an initiator methionine up to a stop codon at positions 244-246. However, somewhere in this sequence there is an apparent anomaly such that the sequence encoding the RXXR cleavage site (nucleotides at  
35 positions 257-268) and the sequence corresponding to the mature persephin protein (positions 269-556) are not co-

linear with this open reading frame. Instead, a second reading frame encodes the cleavage site and the mature persephin. The two cogent reading frames are shown in Figure 17.

- 5 Additional sequencing of the rat persephin has also been performed. Rat genomic fragments were amplified by PCR using Klentaq and rat genomic DNA as a template. The forward primer #40266 (5'-AATCCCCAGGACAGGCAGGGAAT; SEQ ID NO:137) corresponding to a region upstream of the
- 10 mouse persephin gene and a reverse primer M3156 (5'-CGGTACCCAGATCTTCAGCCACCACAGCCACAAGC, SEQ ID NO:138) corresponding to a region within the mature rat persephin sequence were used with the following parameters (95°C for 15 sec, 55°C for 15 sec, 68°C for 45 sec x 30
- 15 cycles). The amplified product was kinased with T4 polynucleotide kinase, the ends were blunted with E. coli DNA polymerase I (Klenow fragment), and cloned into BSKS plasmid.

- Nucleotide sequencing was performed to establish the
- 20 sequence of the entire rat persephin gene (SEQ ID NO:134; Figure 18). An open reading frame was found to extend from the sequence coding for an initiator methionine up to a stop codon at positions 244-246 as was seen with murine persephin. As was also seen with murine
- 25 persephin, an anomaly was found to occur between the sequence encoding the initiator methionine and that encoding the cleavage site for the mature rat persephin such that two cogent reading frames exist as indicated in Figure 18. Irrespective of this anomaly, mammalian cells
- 30 express persephin from either the murine or rat full length genomic sequence as illustrated below.

#### Example 13

- This example illustrates the preparation of a
- 35 bacterial expression vector for murine persephin and its

introduction into an *E. Coli* for expression of recombinant mature persephin.

The persephin polynucleotide encoding the mature murine persephin protein which begins 5 amino acids upstream of the first framework Cys residue (SEQ ID NO:80) was cloned into the pET expression vector pET-30a at the Nde I and Bgl II sites. This persephin polynucleotide was generated by PCR using the murine persephin P1 genomic clone as a template. A forward primer M3157 (5'-GGACTATCATATGGCCCACCACCACCACCACCACCACGACGACGACGACAAGGCCTTGGCTGGTTCATGCCGA, SEQ ID NO:139) encoding an Nde I site, 8 histidine residues, and an enterokinase site, and a reverse primer M3156 (5'-CGGTACCCAGATCTTCAGCCACCACAGCCACAAGC, SEQ ID NO:138), which corresponds to the sequence encoding the last 6 amino acid residues of the mature persephin sequence, the stop codon and a Bgl II site, were used. The PCR reaction conditions were 95°C for 15 sec, 55°C for 15 sec, 68°C for 60 sec x 25 cycles. This PCR product was subcloned into the EcoRV site of BSKS plasmid and sequenced to verify that it contained no mutations. The persephin sequence was then excised from this vector using Nde I and Bgl II and cloned into the Nde I (5') and Bgl II (3') sites of the bacterial expression vector pET30a (Novagen, Madison, WI). This expression vector would, therefore, produce the mature form of the persephin protein possessing an amino terminal tag consisting of 8 histidine residues followed directly by an enterokinase site.

The plasmid was introduced into *E.coli* strain BL21 (DE3). To produce persephin, bacteria harboring this plasmid were grown for 16 hr, harvested, and lysed using 6M guanidine-HCl, 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, 0.01 M Tris at pH 8.0, and recombinant persephin protein was purified from these lysates via chromatography over a Ni-NTA resin (Qiagen).

The protein was eluted using 3 column volumes of Buffer E containing 8 M urea, 0.1 M  $\text{NaH}_2\text{PO}_4$ , 0.01 M Tris, at pH 4.5. The persephin was then renatured by dialysis in renaturation buffer consisting of 0.1 M  $\text{NaH}_2\text{PO}_4$ , 0.01 M Tris at pH 8.3, 0.15 M NaCl, 3 mM cysteine, 0.02% Tween-20, 10% glycerol and containing decreasing concentrations of urea beginning with 4 M for 16 hr, followed by 2 M for 16 hr, 1M for 72 hr, and 0.5 M for 16 hr. The persephin concentration was then determined using a Dot Metric assay (Geno Technology, St. Louis, MO) and stored at 4°C.

This bacterially produced recombinant persephin was used as an immunogen in rabbits to produce antibodies to mature persephin. All of the immunogen injections and blood drawing were performed at Cal Tag Inc. (Healdsburg, CA). The anti-persephin antiserum was demonstrated to specifically recognize persephin, but not neurturin or GDNF, using protein blot analysis. This persephin-specific antiserum was then used to detect persephin in lysates prepared from transfected COS cells.

20

#### Example 14

This example illustrates the preparation of mammalian expression vectors containing the murine or rat persephin genes and their incorporation into mammalian cell lines for the production of mature persephin. To construct the murine plasmid, a P1 clone containing the murine persephin gene was used as a template in a PCR assay. Primers were designed such that the resulting polynucleotide would contain the persephin gene extending from the initiator Methionine codon to the stop codon 3' to the mature persephin coding sequence (SEQ ID NO:131). The PCR reaction utilized a forward primer M3175 (5'-TGCTGTCACCATGGCTGCAGGAAGACTTCGGA, SEQ ID NO:140) and reverse primer M3156 (5'-CGGTACCCAGATCTTCAGCCACCACAGCCACAAGC, SEQ ID NO:138). To construct the analogous rat plasmid, rat genomic DNA was

used as a template in a PCR assay. The PCR reaction utilized a forward primer M3175 (5'-TGCTGTCACCATGGCTGCAGGAAGACTTCGGA, SEQ ID NO:140) and reverse primer M3156 (5'-

- 5 CGGTACCCAGATCTTCAGCCACCACAGCCACAAGC, SEQ ID NO:138). Both PCR reactions were carried out using KlenTaq and the following parameters: 95°C for 15 sec, 55°C for 15 sec, 68°C for 45 sec x 25 cycles. The amplified products were kinased with T4 polynucleotide kinase, the ends were  
10 blunted with E. coli DNA polymerase I (Klenow fragment), and cloned into BSKS plasmid. Nucleotide sequencing was performed to verify that the correct clone was obtained. The rat and murine persephin polynucleotides were excised using Sma I and Hind III and each cloned into a Asp718  
15 (blunted) and Hind III sites of the mammalian expression vector pCB6.

- COS monkey cells were transfected with either the rat or murine persephin expression vectors (16 µg per 5 x 10<sup>5</sup> cells) or the non-recombinant vector (pCB6) itself  
20 using the calcium phosphate precipitation method (Chen and Okayama, *Mol Cell Biol* 7:2745-2752, 1987 which is incorporated by reference). Forty eight hr later the cells were lysed in IP buffer containing 50 mM Tris at pH 7.5, 300 mM NaCl, 1% Triton X-100, 1% deoxycholate, 10 mM  
25 EDTA, 0.1% SDS, 5 µg/ml leupeptin, 7 µg/ml pepstatin, and 250 µM PMSF. The samples were loaded onto a 15% SDS-polyacrylamide gel and the proteins were separated by electrophoresis. The proteins were then transferred to nitrocellulose by electroblotting. This nitrocellulose  
30 membrane was incubated with anti-persephin antibodies to detect the presence of persephin in the lysates.

- As is shown in Figure 19, lysates from cells transfected with either the rat or murine persephin expression vectors, but not the lysate from cells  
35 transfected with pCB6, contain high amounts of persephin. The size of the persephin detected was approximately 14



kD which is consistent with the size predicted for the processed, i.e. mature form of persephin. This demonstrates that both the murine and rat persephin genes are capable of directing the synthesis of a properly processed persephin molecule.

#### Example 15

The following example illustrates the methods that can be used for isolation and identification of human persephin.

The identification of murine and rat persephin sequences now allows us to identify and isolate the human persephin gene. Due to the high conservation between human and rodent GDNF (approximately 95% identity) and between human and rodent neurturin (approximately 90% identity), it is believed that a similarly close relationship (i.e. greater than 85% identity) will be present between rodent and human persephin.

Different strategies can be used to obtain the human persephin gene. In one preferred strategy, human genomic and cDNA libraries are screened by hybridization to the murine and/or the rat persephin sequences that have been identified herein (SEQ ID NOS:79-83) or portions of these sequences. These DNA sequences or probes are labeled as described above, for example, with 32P-dCTP using either random priming or polynucleotide kinase. Hybridization conditions are described above and various stringency conditions of hybridization are used. Stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook et al. *supra*.

In an alternate preferred strategy, primers corresponding to portions of the rat or the murine persephin nucleotide sequence (or derivatives thereof)

- are employed in a PCR reaction using either human genomic DNA or cDNA reverse transcribed from RNA isolated from human tissue. As an example, forward primer M2026 (SEQ ID NO:96) and reverse primer, M3028 (SEQ ID NO:101) can
- 5 be used in a PCR reaction using various conditions as described above and human DNA templates to amplify a human persephin fragment. Primers that amplify such a fragment as confirmed by nucleotide sequencing of the fragment are then used to obtain human persephin clones.
- 10 The clones are identified by virtue of their producing the same amplified fragment following PCR with the selected primers in a human genomic library in a  $\phi$ 1 bacteriophage vector (library screening service of Genome Systems, Inc., St. Louis, MO).
- 15 After obtaining positive clones by either of the methods above, these human persephin clones are isolated and fragments are subcloned into Bluescript KS plasmids and sequenced. Nucleotide sequencing is performed using fluorescent dye terminator technology on an Applied
- 20 Biosystems automated sequencer Model #373 (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Plasmid DNA for sequencing is prepared using the Wizard Miniprep kit (Promega Corp., Madison, WI) according to manufacturer's instructions. Sequences
- 25 of these human fragments which are orthologous to the rat and murine persephin sequences are then identified and the full length nucleotide sequence of human persephin is established from the sequences of these fragments.

#### Example 16

- 30 This example illustrates the preparation of chimeric or hybrid polypeptide molecules that contain portions derived from persephin (PSP) and portions derived from neurturin (NTN).
- 35 As closely related members of the TGF $\beta$  family, each of persephin and neurturin is predicted to have a very

similar overall structure, yet while neurturin promotes the survival of sympathetic neurons, the closely related persephin does not. Two chimeras were produced by essentially replacing portions of persephin with

5 neurturin, with the crossover point located between the two adjacent, highly conserved third and fourth cysteine residues. The first chimera, named PSP/NTN (SEQ ID NO:141, Figure 20), contains the first 63 residues of

10 mature murine persephin combined with residues 68 through 100 of mature murine neurturin (using E. coli preferred codons). To construct this molecule, two PCR reactions were performed: 1) using the forward primer M2012 (5'-TAATACGACTCACTATAGGGGAA, SEQ ID NO:142) and reverse

15 primer M2188 (5'-TCGTCTTCGTAAGCAGTCGGACGGCAGCAGGGTCGGCCATGGGCTCGAC, SEQ ID NO:143) and the pET30a-murine persephin plasmid as template (see Example 13); and 2) using the forward

20 primer M2190 (5'-TGCTGCCGTCGACTGCTTACGAAGACGA, SEQ ID NO:144) and reverse primer M2186 (5'-GTTATGCTAGTTATTGCTCAGCGGT, SEQ ID NO:145) and the pET30a-murine (E.coli preferred codons) neurturin plasmid as

25 template (see Example 6). Both PCR reactions were carried out using the following parameters: 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec x 25 cycles. The

30 products of these two PCR reactions were gel purified, mixed together, and a PCR reaction was performed under the following conditions: 94°C for 30 sec, 60°C for 20 min, 68°C for 5 min. After 8 cycles, an aliquot of this

35 reaction was used as template in a third PCR reaction using the forward primer M2012 and reverse primer M2186 under the following conditions: 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec x 25 cycles. The resulting product was kinased with T4 polynucleotide kinase, the ends were blunted with E. coli DNA polymerase I (Klenow fragment), and cloned into BSKS plasmid. Nucleotide sequencing was performed to verify that the correct clone

was obtained. The PSP/NTN fragment was excised using Nde I and Bam HI and cloned into the corresponding sites of the bacterial expression vector pET30a.

The second chimera, named NTN/PSP (SEQ ID NO:146, Figure 20), encodes the converse molecule. It contains the first 67 residues of mature murine neurturin (using E.coli preferred codons) combined with residues 64 to 96 of mature murine persephin. To construct this molecule, we performed two PCR reactions: 1) using the forward primer M2012 and reverse primer M2183 (5'-CACATCAGCATAGCTGGTGGGCTGGCAGCACGGGTGAGCACGAGCACGTT, SEQ ID NO:147) and the pET30a-murine (E.coli preferred codons) neurturin plasmid as template; and 2) using the forward primer M2187 (5'-TGCTGCCAGCCCACCAGCTATGCTG, SEQ ID NO:148) and reverse primer M2186 (5'-GTTATGCTAGTTATTGCTCAGCGGT, SEQ ID NO:145) and the pET30a-murine persephin plasmid as template. Both PCR reactions were carried out using the following parameters: 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec x 25 cycles. The products of these two PCR reactions were used to construct the final NTN/PSP pET30a plasmid as detailed above for PSP/NTN except that Bgl II was used instead of Bam HI. These chimeric proteins were produced in E.coli and purified by Ni-NTA chromatography as described above (Example 13).

The purified proteins were assayed for their ability to promote survival in the SCG sympathetic neuron assay. The NTN/PSP protein did not promote survival, whereas the PSP/NTN protein promoted the survival of sympathetic neurons similar to that observed for neurturin itself. These results indicate that neurturin residues lying downstream of the 2 adjacent, highly conserved cysteine residues are critical for activity in promoting survival in SCG sympathetic neurons. In contrast, the corresponding residues of persephin are not sufficient for promoting survival in sympathetic neurons.

## Example 17

This example illustrates the neuronal survival promoting activity of persephin in mesencephalic cells.

The profile of survival promoting activity of  
5 persephin is different from that of neurturin and GDNF.  
In contrast to the survival promoting activity produced  
by neurturin and GDNF in sympathetic and sensory neurons,  
persephin showed no survival promoting activity in these  
tissues. We further evaluated the neuronal survival  
10 promoting activity of persephin in mesencephalic cells.

Timed-pregnant Sprague-Dawley rats were purchased  
from Harlan Sprague-Dawley. The mesencephalon was taken  
from rats measuring 1.2 to 1.4 cm in length and time  
dated to be embryonic day 14. The cranium was removed  
15 and the entire mesencephalon was placed in cold L15. The  
pooled mesencephalic tissue was resuspended in a serum-  
free medium consisting of DME/Hams F12 (#11330-032, Life  
Technologies) 1 mg/ml BSA, Fraction V (A-6793, Sigma  
Chemical Co.), 5  $\mu$ M Insulin (I-5500, Sigma), 10 nM  
20 progesterone (P0130, Sigma), 100  $\mu$ M putrescine, (p7505,  
Sigma), 30 nM Selenium (SO7150, Pflatz & Bauer), 10 ng/ml  
rat transferrin (012-000-050, Jackson Chrompure), 100  
U/ml penicillin, and 100 U/ml of streptomycin. The  
pooled mesencephalic tissues were triturated  
25 approximately 80 times using a bent-tip pipette and the  
cells were plated in a 24-well dish (CoStar) at a density  
of 15,000 cells in a 100- $\mu$ l drop. The dishes were coated  
with 125 ng/ml poly-d-lysine (p-7280, Sigma) and 25 ng/ml  
laminin (#40232, Collaborative Biomedical Products).  
30 These dissociated cells were allowed to attach for 2  
hours at 37°C in 5% CO<sub>2</sub> and then fed with another 500  $\mu$ l  
of the above serum-free medium with or without  
approximately 100 ng/ml of recombinant Persephin. These  
cells were photographed after 3 days of culture.  
35 Inspection of the cells over the course of 3 days in  
culture, showed a gradual decrease in cell number. In

the absence of any growth factor, almost all of the cells were dead (Figure 21A). In the presence of persephin, a large increase in mesencephalic neuronal cell survival was evident (Figure 21B).

5

#### Example 18

This example illustrates the expression of persephin in various tissues.

A survey of persephin expression was performed in  
10 adult mouse tissues using semi-quantitative RT/PCR (see Example 9). Poly A RNA was isolated from brain, cerebellum, kidney, lung, heart, ovary, sciatic nerve, dorsal root ganglia, blood and spleen. This was then reverse transcribed to produce cDNA (see Kotzbauer et al.  
15 Nature 384:467-470, 1996 which is incorporated by reference). The PCR primers used were as follows: forward primer: 5'-CCTCGGAGGAGAAGGTCATCTTC (SEQ ID NO:149) and reverse primer: 5'TCATCAAGGAAGGTCACATCAGCATA (SEQ ID NO:101). PCR was done for 26 cycles with an  
20 annealing temperature of 60°C. To control for the presence of genomic DNA, RNA samples which were not reverse transcribed were used for PCR (for example, the tissue control shown in figure 22 is labeled "Kidney no RT"). All the samples were found to be without genomic  
25 DNA contamination.

As shown in Figure 22, a band of the correct size (160 bp) was seen in the kidney sample. At higher cycle numbers a persephin band was also seen in brain. Thus, the distribution of expression of persephin in various  
30 mouse tissues differs from that of neurturin in rat (Example 8).

Deposit of Strain. The following strain is on deposit under the terms of the Budapest Treaty, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville,  
35 MD. The accession number indicated was assigned after successful viability testing, and the requisite fees were

paid. Access to said cultures will be available during  
pendency of the patent application to one determined by  
the Commissioner to be entitled thereto under 37 CFR 1.14  
and 35 USC 122. All restriction on availability of said  
5 cultures to the public will be irrevocably removed upon  
the granting of a patent based upon the application.  
Moreover, the designated deposits will be maintained for  
a period of thirty (30) years from the date of deposit,  
or for five (5) years after the last request for the  
10 deposit, or for the enforceable life of the U.S. patent,  
whichever is longer. Should a culture become nonviable  
or be inadvertently destroyed, or, in the case of  
plasmid-containing strains, lose its plasmid, it will be  
replaced with a viable culture. The deposited materials  
15 mentioned herein are intended for convenience only, and  
are not required to practice the present invention in  
view of the description herein, and in addition, these  
materials are incorporated herein by reference.

20	<hr/>	<hr/>	<hr/>
	Strain	Deposit Date	ATCC No.
	<hr/>	<hr/>	<hr/>
25	DG44CHO-pHSP-NGFI-B	August 25, 1995	CRL 11977
	<hr/>	<hr/>	<hr/>

In view of the above, it will be seen that the  
several advantages of the invention are achieved and  
other advantageous results attained.

30 As various changes could be made in the above  
methods and compositions without departing from the scope  
of the invention, it is intended that all matter  
contained in the above description and shown in the  
accompanying drawings shall be interpreted as  
35 illustrative and not in a limiting sense.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: JOHNSON JR., EUGENE M.  
MILBRANDT, JEFFREY D.  
KOTZBAUER, PAUL T.  
LAMPE, PATRICIA A.
- (ii) TITLE OF INVENTION: PERSEPHIN AND RELATED GROWTH FACTORS
- (iii) NUMBER OF SEQUENCES: 176
- (iv) CORRESPONDENCE ADDRESS:
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  - (D) STATE: MISSOURI
  - (E) COUNTRY: US
  - (F) ZIP: 63105-1817
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: HOLLAND, DONALD R.
  - (B) REGISTRATION NUMBER: 35,197
  - (C) REFERENCE/DOCKET NUMBER: 976163
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (314) 727-5188
  - (B) TELEFAX: (314) 727-6092

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 102 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  

Ala	Arg	Leu	Gly	Ala	Arg	Pro	Cys	Gly	Leu	Arg	Glu	Leu	Glu	Val	Arg
1				5					10					15	
Val	Ser	Glu	Leu	Gly	Leu	Gly	Tyr	Ala	Ser	Asp	Glu	Thr	Val	Leu	Phe
			20				25						30		
Arg	Tyr	Cys	Ala	Gly	Ala	Cys	Glu	Ala	Ala	Ala	Arg	Val	Tyr	Asp	Leu
		35				40						45			
Gly	Leu	Arg	Arg	Leu	Arg	Gln	Arg	Arg	Arg	Leu	Arg	Arg	Glu	Arg	Val
		50				55							60		



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Arg Ala Gln Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser  
 65 70 75 80  
 Phe Leu Asp Ala His Ser Arg Tyr His Thr Val His Glu Leu Ser Ala  
 85 90 95  
 Arg Glu Cys Ala Cys Val  
 100

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 100 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Pro Gly Ala Arg Pro Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser  
 1 5 10 15  
 Glu Leu Gly Leu Gly Tyr Thr Ser Asp Glu Thr Val Leu Phe Arg Tyr  
 20 25 30  
 Cys Ala Gly Ala Cys Glu Ala Ala Ile Arg Ile Tyr Asp Leu Gly Leu  
 35 40 45  
 Arg Arg Leu Arg Gln Arg Arg Arg Val Arg Arg Glu Arg Ala Arg Ala  
 50 55 60  
 His Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu  
 65 70 75 80  
 Asp Val His Ser Arg Tyr His Thr Leu Gln Glu Leu Ser Ala Arg Glu  
 85 90 95  
 Cys Ala Cys Val  
 100

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 16 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 6  
 (D) OTHER INFORMATION: /note= "ANY AMINO ACID"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser Gly Ala Arg Pro Xaa Gly Leu Arg Glu Leu Glu Val Ser Val Ser  
 1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:4:

124

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 1
  - (D) OTHER INFORMATION: /note= "ANY AMINO ACID"
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 6
  - (D) OTHER INFORMATION: /note= "SERINE OR CYSTEINE"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  

Xaa	Cys	Ala	Gly	Ala	Xaa	Glu	Ala	Ala	Val
1				5					10
- (2) INFORMATION FOR SEQ ID NO:5:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS:
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 1
    - (D) OTHER INFORMATION: /note= "ANY AMINO ACID"
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 2
    - (D) OTHER INFORMATION: /note= "ANY AMINO ACID"
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 17
    - (D) OTHER INFORMATION: /note= "GLUTAMINE OR GLUTAMIC ACID"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  

Xaa	Xaa	Val	Glu	Ala	Lys	Pro	Cys	Cys	Gly	Pro	Thr	Ala	Tyr	Glu	Asp
1				5					10					15	
Xaa Val Ser Phe Leu Ser Val															
20															
- (2) INFORMATION FOR SEQ ID NO:6:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 10 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS:
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide

125

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Tyr His Thr Leu Gln Glu Leu Ser Ala Arg  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 197 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Gln Arg Trp Lys Ala Ala Ala Leu Ala Ser Val Leu Cys Ser Ser  
 1 5 10 15  
 Val Leu Ser Ile Trp Met Cys Arg Glu Gly Leu Leu Leu Ser His Arg  
 20 25 30  
 Leu Gly Pro Ala Leu Val Pro Leu His Arg Leu Pro Arg Thr Leu Asp  
 35 40 45  
 Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala  
 50 55 60  
 Pro Asp Ala Met Glu Leu Arg Glu Leu Thr Pro Trp Ala Gly Arg Pro  
 65 70 75 80  
 Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg Ala  
 85 90 95  
 Arg Leu Gly Ala Arg Pro Cys Gly Leu Arg Glu Leu Glu Val Arg Val  
 100 105 110  
 Ser Glu Leu Gly Leu Gly Tyr Ala Ser Asp Glu Thr Val Leu Phe Arg  
 115 120 125  
 Tyr Cys Ala Gly Ala Cys Glu Ala Ala Ala Arg Val Tyr Asp Leu Gly  
 130 135 140  
 Leu Arg Arg Leu Arg Gln Arg Arg Arg Leu Arg Arg Glu Arg Val Arg  
 145 150 155 160  
 Ala Gln Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe  
 165 170 175  
 Leu Asp Ala His Ser Arg Tyr His Thr Val His Glu Leu Ser Ala Arg  
 180 185 190  
 Glu Cys Ala Cys Val  
 195

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

126

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Arg Arg Trp Lys Ala Ala Ala Leu Val Ser Leu Ile Cys Ser Ser  
 1 5 10 15  
 Leu Leu Ser Val Trp Met Cys Gln Glu Gly Leu Leu Leu Gly His Arg  
 20 25 30  
 Leu Gly Pro Ala Leu Ala Pro Leu Arg Arg Pro Pro Arg Thr Leu Asp  
 35 40 45  
 Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala  
 50 55 60  
 Pro Asp Ala Val Glu Leu Arg Glu Leu Ser Pro Trp Ala Ala Arg Ile  
 65 70 75 80  
 Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg Pro  
 85 90 95  
 Gly Ala Arg Pro Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu  
 100 105 110  
 Leu Gly Leu Gly Tyr Thr Ser Asp Glu Thr Val Leu Phe Arg Tyr Cys  
 115 120 125  
 Ala Gly Ala Cys Glu Ala Ala Ile Arg Ile Tyr Asp Leu Gly Leu Arg  
 130 135 140  
 Arg Leu Arg Gln Arg Arg Arg Val Arg Arg Glu Arg Ala Arg Ala His  
 145 150 155 160  
 Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp  
 165 170 175  
 Val His Ser Arg Tyr His Thr Leu Gln Glu Leu Ser Ala Arg Glu Cys  
 180 185 190  
 Ala Cys Val  
 195

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCGCGGTTGG GGGCGCGGCC TTGCGGGCTG CGCGAGCTGG AGGTGCGCGT GAGCGAGCTG 60  
 GGCCTGGGCT ACGCGTCCGA CGAGACGGTG CTGTTCCGCT ACTGCGCAGG CGCCTGCGAG 120  
 GCTGCCGCGC GCGTCTACGA CCTCGGGCTG CGACGACTGC GCCAGCGGCG GCGCCTGCGG 180  
 CGGGAGCGGG TGCGCGCGCA GCCCTGCTGC CGCCCAGCGG CCTACGAGGA CGAGGTGTCC 240  
 TTCCTGGACG CGCACAGCCG CTACCACAGC GTGCACGAGC TGTCGGCGCG CGAGTGCGCC 300  
 TGCCTG 306

127

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 300 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCGGGGGCTC GGCCTTGTGG GCTGCGCGAG CTCGAGGTGC GCGTGAGCGA GCTGGGCCTG	60
GGCTACACGT CGGATGAGAC CGTGCTGTTC CGCTACTGCG CAGGCGCGTG CGAGGCGGCC	120
ATCCGCATCT ACGACCTGGG CCTTCGGCGC CTGCGCCAGC GGAGGCGCGT GCGCAGAGAG	180
CGGGCGCGGG CGCACCCGTG TTGTCGCCCC ACGGCCTATG AGGACGAGGT GTCCTTCCTG	240
GACGTGCACA GCCGCTACCA CACGCTGCAA GAGCTGTCGG CGCGGGAGTG CGCGTGCGTG	300

## (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 591 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGCAGCGCT GGAAGGCGGC GGCCTTGGCC TCAGTGCTCT GCAGCTCCGT GCTGTCCATC	60
TGGATGTGTC GAGAGGGCCT GCTTCTCAGC CACCGCCTCG GACCTGCGCT GGTCCCCCTG	120
CACCGCCTGC CTCGAACCCT GGACGCCCCG ATTGCCCCGCC TGGCCAGTA CCGTGCACTC	180
CTGCAGGGGG CCCC GGATGC GATGGAGCTG CGCGAGCTGA CGCCCTGGGC TGGGCGGCCC	240
CCAGGTCCGC GCCGTCGGGC GGGGCCCCGG CGGCGGCGCG CGCGTGCGCG GTTGGGGGCG	300
CGGCCTTGCG GGCTGCGCGA GCTGGAGGTG CGCGTGAGCG AGCTGGGCCT GGGCTACGCG	360
TCCGACGAGA CGGTGCTGTT CCGCTACTGC GCAGGCGCCT GCGAGGCTGC CGCGCGCGTC	420
TACGACCTCG GGCTGCGACG ACTGCGCCAG CGGCGGCGCC TCGGCGGGGA GCGGGTGC GC	480
GCGCAGCCCT GCTGCCGCCC GACGGCCTAC GAGGACGAGG TGTCTTCCT GGACGCGCAC	540
AGCCGCTACC ACACGGTGCA CGAGCTGTCG GCGCGCGAGT GCGCCTGCGT G	591

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 585 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGAGGCGCT GGAAGGCAGC GGCCCTGGTG TCGCTCATCT GCAGCTCCCT GCTATCTGTC	60
TGGATGTGCC AGGAGGGTCT GCTCTTGGGC CACCGCCTGG GACCCGCGCT TGCCCGCTA	120
CGACGCCCTC CACGCACCCT GGACGCCCGC ATCGCCCGCC TGGCCAGTA TCGCGCTCTG	180
CTCCAGGGCG CCCCCGACGC GGTGGAGCTT CGAGAACTTT CTCCTGGGC TGCCCGCATC	240
CCGGGACCGC GCCGTCGAGC GGGTCCCCGG CGTCGGCGGG CGCGGCCGGG GGCTCGGCCT	300
TGTGGGCTGC GCGAGCTCGA GGTGCGCGTG AGCGAGCTGG GCCTGGGCTA CACGTCGGAT	360
GAGACCGTGC TGTTCCGCTA CTGCGCAGGC GCGTGCAGG CGGCCATCCG CATCTACGAC	420
CTGGGCCTTC GCGCCTGCG CCAGCGGAGG CGCGTGCACA GAGAGCGGGC GCGGGCGCAC	480
CCGTGTTGTC GCCCGACGGC CTATGAGGAC GAGGTGTCCT TCCTGGACGT GCACAGCCGC	540
TACCACACGC TGCAAGAGCT GTCGGCGCGG GAGTGCGCGT GCGTG	585

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 348 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGAGGGAGAG CGCGCGGTGG TTTCGTCCGT GTGCCCCGCG CCCGGCGCTC CTCGCGTGGC	60
CCCGCGTCCT GAGCGCGCTC CAGCCTCCCA CGCGCGCCAC CCCGGGGTTC ACTGAGCCCG	120
GCGAGCCCGG GGAAGACAGA GAAAGAGAGG CCAGGGGGGG AACCCCATGG CCCGGCCCGT	180
GTCCCGCACC CTGTGCGGTG GCCTCCTCCG GCACGGGGTC CCCGGGTGCG CTCGGTCCCC	240
CGCGATCCGG ATGGCGCAGC CAGTGGCTGG GGCCGGGCCG GGCTCGGGTG GTCGGAGGAG	300
TCACCACTGA CCGGTCATC TGGAGCCCGT GGCAGGCCGA GGCCCAGG	348

## (2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 87 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGCTACCTCA CGCCCCCGA CCTGCGAAAG GGCCCTCCCT GCCGACCCTC GCTGAGAACT	60
GACTTCACAT AAAGTGTTGG AACTCCC	87

## (2) INFORMATION FOR SEQ ID NO:15:

129

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Gln Arg Trp Lys Ala Ala Ala Leu Ala Ser Val Leu Cys Ser Ser  
1 5 10 15  
Val Leu Ser

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Arg Arg Trp Lys Ala Ala Ala Leu Val Ser Leu Ile Cys Ser Ser  
1 5 10 15  
Leu Leu Ser

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGCAGCGCT GGAAGGCGGC GGCCTTGGCC TCAGTGCTCT GCAGCTCCGT GCTGTCC

57

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATGAGGCGCT GGAAGGCAGC GGCCCTGGTG TCGCTCATCT GCAGCTCCCT GCTATCT

57

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## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 76 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Ile Trp Met Cys Arg Glu Gly Leu Leu Leu Ser His Arg Leu Gly Pro
1      5      10      15
Ala Leu Val Pro Leu His Arg Leu Pro Arg Thr Leu Asp Ala Arg Ile
20     25     30
Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala Pro Asp Ala
35     40     45
Met Glu Leu Arg Glu Leu Thr Pro Trp Ala Gly Arg Pro Pro Gly Pro
50     55     60
Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg
65     70     75

```

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 228 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

ATCTGGATGT GTCGAGAGGG CCTGCTTCTC AGCCACCGCC TCGGACCTGC GCTGGTCCCC      60
CTGCACCGCC TGCCTCGAAC CCTGGACGCC CGGATTGCCC GCCTGGCCCA GTACCGTGCA      120
CTCCTGCAGG GGGCCCCGGA TGCGATGGAG CTGCGCGAGC TGACGCCCTG GGCTGGGCGG      180
CCCCCAGGTC CGCGCCGTCG GCGGGGGCCC CGGCGGCGGC GCGCGCGT      228

```

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 228 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

GTCTGGATGT GCCAGGAGGG TCTGCTCTTG GGCACCGCC TGGGACCCGC GCTTGCCCCG      60
CTACGACGCC CTCCACGCAC CCTGGACGCC CGCATCGCCC GCCTGGCCCA GTATCGCGCT      120

```



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CTGCTCCAGG GCGCCCCCGA CGCGGTGGAG CTTCGAGAAC TTTCTCCCTG GGCTGCCCGC 180  
 ATCCCGGGAC CGCGCCGTCG AGCGGGTCCC CGGCGTCGGC GGGCGCGG 228

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 76 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Trp Met Cys Gln Glu Gly Leu Leu Leu Gly His Arg Leu Gly Pro  
 1 5 10 15  
 Ala Leu Ala Pro Leu Arg Arg Pro Pro Arg Thr Leu Asp Ala Arg Ile  
 20 25 30  
 Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala Pro Asp Ala  
 35 40 45  
 Val Glu Leu Arg Glu Leu Ser Pro Trp Ala Ala Arg Ile Pro Gly Pro  
 50 55 60  
 Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg  
 65 70 75

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 95 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Gln Arg Trp Lys Ala Ala Ala Leu Ala Ser Val Leu Cys Ser Ser  
 1 5 10 15  
 Val Leu Ser Ile Trp Met Cys Arg Glu Gly Leu Leu Leu Ser His Arg  
 20 25 30  
 Leu Gly Pro Ala Leu Val Pro Leu His Arg Leu Pro Arg Thr Leu Asp  
 35 40 45  
 Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala  
 50 55 60  
 Pro Asp Ala Met Glu Leu Arg Glu Leu Thr Pro Trp Ala Gly Arg Pro  
 65 70 75 80  
 Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg  
 85 90 95

## (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 95 amino acids

132

- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

Met Arg Arg Trp Lys Ala Ala Ala Leu Val Ser Leu Ile Cys Ser Ser
 1      5      10      15
Leu Leu Ser Val Trp Met Cys Gln Glu Gly Leu Leu Leu Gly His Arg
      20      25      30
Leu Gly Pro Ala Leu Ala Pro Leu Arg Arg Pro Pro Arg Thr Leu Asp
      35      40      45
Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu Leu Gln Gly Ala
      50      55      60
Pro Asp Ala Val Glu Leu Arg Glu Leu Ser Pro Trp Ala Ala Arg Ile
      65      70      75      80
Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg
      85      90      95

```

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 285 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```

ATGCAGCGCT GGAAGGCGGC GGCCTTGGCC TCAGTGCTCT GCAGCTCCGT GCTGTCCATC      60
TGGATGTGTC GAGAGGGCCT GCTTCTCAGC CACCGCCTCG GACCTGCGCT GGTCCCCCTG      120
CACCGCCTGC CTCGAACCCT GGACGCCCCG ATTGCCCCGC TGGCCCAGTA CCGTGCACTC      180
CTGCAGGGGG CCCC GGATGC GATGGAGCTG CGCGAGCTGA CGCCCTGGGC TGGGCGGCCC      240
CCAGGTCCGC GCCGTCGGGC GGGGCCCCGG CGGCGGCGCG CGCGT      285

```

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 285 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```

ATGAGGCGCT GGAAGGCAGC GGCCCTGGTG TCGCTCATCT GCAGCTCCCT GCTATCTGTC      60
TGGATGTGCC AGGAGGGTCT GCTCTTGGGC CACCGCCTGG GACCCGCGCT TGCCCCGCTA      120

```

133

CGACGCCCTC CACGCACCTT GGACGCCCGC ATCGCCCGCC TGGCCAGTA TCGCGCTCTG 180  
CTCCAGGGCG CCCCCGACGC GGTGGAGCTT CGAGAACTTT CTCCCTGGGC TGCCCGCATC 240  
CCGGGACCGC GCCGTCGAGC GGGTCCCCGG CGTCGGCGGG CGCGG 285

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 169 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGCAGCGCT GGAAGGCGGC GGCCTTGCC TCAGTGCTCT GCAGCTCCGT GCTGTCCATC 60  
TGGATGTGTC GAGAGGGCCT GCTTCTCAGC CACCGCCTCG GACCTGCGCT GGTCCCCCTG 120  
CACCGCCTGC CTCGAACCCT GGACGCCCGG ATTGCCCGCC TGGCCAGT 169

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 425 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ACCGTGCACT CCTGCAGGGG GCCCCGATG CGATGGAGCT GCGCGAGCTG ACGCCCTGGG 60  
CTGGGCGGCC CCCAGGTCCG CGCCGTCGGG CGGGGCCCCG GCGGCGGCGC GCGCGTGCGC 120  
GGTTGGGGGC GCGGCCTTGC GGGCTGCGCG AGCTGGAGGT GCGCGTGAGC GAGCTGGGCC 180  
TGGGCTACGC GTCCGACGAG ACGGTGCTGT TCCGCTACTG CGCAGGCGCC TGCAGGGCTG 240  
CCGCGCGCGT CTACGACCTC GGGCTGCGAC GACTGCGCCA GCGGCGGCGC CTGCGGCGGG 300  
AGCGGGTGCG CGCGCAGCCC TGCTGCCGCC CGACGGCCTA CGAGGACGAG GTGTCCTTCC 360  
TGGACGCGCA CAGCCGCTAC CACACGGTGC ACGAGCTGTC GCGCGCGGAG TCGCCTGCG 420  
TGTA 425

## (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 169 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

134

ATGAGGCGCT GGAAGGCAGC GGCCCTGGTG TCGCTCATCT GCAGCTCCCT GCTATCTGTC 60  
 TGGATGTGCC AGGAGGGTCT GCTCTTGGGC CACCGCCTGG GACCCGCGCT TGCCCCGCTA 120  
 CGACGCCCTC CACGCACCCT GGACGCCCCG ATCGCCCGCC TGGCCCACT 169

## (2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 419 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATCGCGCTCT GCTCCAGGGC GCGCCCGACG CGGTGGAGCT TCGAGAACTT TCTCCCTGGG 60  
 CTGCCCCGAT CCGGGGACCG CGCCGTCGAG CGGGTCCCCG GCGTCGGCGG GCGCGGCCCG 120  
 GGGCTCGGCC TTGTGGGCTG CGCGAGCTCG AGGTGCGCGT GAGCGAGCTG GGCCTGGGCT 180  
 ACACGTCGGA TGAGACCGTG CTGTTCCGCT ACTGCGCAGG CGCGTGCAGG GCGGCCATCC 240  
 GCATCTACGA CCTGGGCCTT CGGCGCCTGC GCCAGCGGAG GCGCGTGCAG AGAGAGCGGG 300  
 CGCGGGCGCA CCCGTGTTGT CGCCCGACGG CCTATGAGGA CGAGGTGTCC TTCCTGGACG 360  
 TGCACAGCCG CTACCACACG CTGCAAGAGC TGTCGGCGCG GGAGTGCAGG TCGTGTGA 419

## (2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 94 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu Leu Gly Leu Gly  
 1 5 10 15  
 Tyr Ala Ser Asp Glu Thr Val Leu Phe Arg Tyr Cys Ala Gly Ala Cys  
 20 25 30  
 Glu Ala Ala Ala Arg Val Tyr Asp Leu Gly Leu Arg Arg Leu Arg Gln  
 35 40 45  
 Arg Arg Arg Leu Arg Arg Glu Arg Val Arg Ala Gln Pro Cys Cys Arg  
 50 55 60  
 Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp Ala His Ser Arg  
 65 70 75 80  
 Tyr His Thr Val His Glu Leu Ser Ala Arg Glu Cys Ala Cys  
 85 90

## (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:

135

- (A) LENGTH: 94 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```

Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu Leu Gly Leu Gly
1      5      10      15
Tyr Thr Ser Asp Glu Thr Val Leu Phe Arg Tyr Cys Ala Gly Ala Cys
20      25      30
Glu Ala Ala Ile Arg Ile Tyr Asp Leu Gly Leu Arg Arg Leu Arg Gln
35      40      45
Arg Arg Arg Val Arg Arg Glu Arg Ala Arg Ala His Pro Cys Cys Arg
50      55      60
Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp Val His Ser Arg
65      70      75      80
Tyr His Thr Leu Gln Glu Leu Ser Ala Arg Glu Cys Ala Cys
85      90

```

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 8 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 2
  - (D) OTHER INFORMATION: /note= "SERINE OR THREONINE"

- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```

Val Xaa Xaa Leu Gly Leu Gly Tyr
1      5

```

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

136

- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 2
  - (D) OTHER INFORMATION: /note= "THREONINE OR GLUTAMIC ACID"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION: /note= "VALINE OR LEUCINE"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 4
  - (D) OTHER INFORMATION: /note= "LEUCINE OR ISOLEUCINE"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 9
  - (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 11
  - (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 13
  - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC ACID"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 14
  - (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
- |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Xaa | Xaa | Xaa | Phe | Arg | Tyr | Cys | Xaa | Gly | Xaa | Cys | Xaa | Xaa | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
- (2) INFORMATION FOR SEQ ID NO:35:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 5
  - (D) OTHER INFORMATION: /note= "THREONINE OR VALINE OR ISOLEUCINE"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 7
  - (D) OTHER INFORMATION: /note= "TYROSINE OR PHENYLALANINE"
- (ix) FEATURE:
- (A) NAME/KEY: Modified-site
  - (B) LOCATION: 8
  - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC ACID"

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(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 10  
    (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC  
ACID"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 11  
    (D) OTHER INFORMATION: /note= "VALINE OR LEUCINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Cys	Cys	Arg	Pro	Xaa	Ala	Xaa	Xaa	Asp	Xaa	Xaa	Ser	Phe	Leu	Asp
1				5					10				15	

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 11 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS:  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 5  
    (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 7  
    (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 9  
    (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC  
ACID"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 10  
    (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Phe	Arg	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa	Xaa	Ala
1				5					10	

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 11 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS:  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 5

138

(D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 7

(D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 9

ACID" (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 10

(D) OTHER INFORMATION: /note= "SERINE OR ALANINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Phe	Arg	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa	Xaa	Ala
1				5					10	

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 5

VALINE" (D) OTHER INFORMATION: /note= "ISOLEUCINE OR THREONINE OR

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 7

(D) OTHER INFORMATION: /note= "TYROSINE OR PHENYLALANINE"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 8

ACID" (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 10

ACID" (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Cys	Cys	Arg	Pro	Xaa	Ala	Xaa	Xaa	Asp	Xaa
1				5					10

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid



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- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 2
  - (D) OTHER INFORMATION: /note= "TYROSINE OR PHENYLALANINE"
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC ACID"
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 5
  - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC ACID"
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 6
  - (D) OTHER INFORMATION: /note= "VALINE OR LEUCINE"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:  
Ala Xaa Xaa Asp Xaa Xaa Ser Phe Leu Asp  
1 5 10
- (2) INFORMATION FOR SEQ ID NO:40:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 8 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS:
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 2
    - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR THREONINE"
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 3
    - (D) OTHER INFORMATION: /note= "LEUCINE OR VALINE"
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 4
    - (D) OTHER INFORMATION: /note= "ISOLEUCINE OR LEUCINE"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:  
Glu Xaa Xaa Xaa Phe Arg Tyr Cys  
1 5
- (2) INFORMATION FOR SEQ ID NO:41:
  - (i) SEQUENCE CHARACTERISTICS:

140

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR THREONINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "LEUCINE OR VALINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "ISOLEUCINE OR LEUCINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC

ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Glu Xaa Xaa Xaa Phe Arg Tyr Cys Xaa Gly Xaa Cys Xaa  
 1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GTNWSNGANY TNGGNYTNGG NTA

23

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

141

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TTYMGNTAYT GYDSNGGNDG NTGYGANKCN GC

32

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GCNGMNTCRC ANSHNCCNSH RCARTANCKR AA

32

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

TCRTCNTCRW ANGCMRYNGG NCKRCARCA

29

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TCNARRAANS WNAVNTCRTC NTCRWANGC

29

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

142

GARRMNBTHN TNTTYMGNTA YTG

23

## (2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 38 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GARRMNBTHN TNTTYMGNTA YTGYSNGGN DSNTGHGA

38

## (2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ser	Gly	Ala	Arg	Pro	Xaa	Gly	Leu	Arg	Glu	Leu	Glu	Val	Ser	Val	Ser
1				5					10					15	

## (2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCNACNGCNT AYGARGA

17

## (2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Ala	Arg	Ala	His	Pro	Cys	Cys	Arg	Pro	Thr	Ala	Tyr	Glu	Asp	Glu	Val
1				5					10					15	

143

Ser Phe Leu Asp  
20

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ARYTCYTGNA RNGTRTGRTA

20

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GACGAGGTGT CCTTCCTGGA CGTACACA

28

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TAGCGGCTGT GTACGTCCAG GAAGGACACC TCGT

34

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CAGCGACGAC GCGTGCGCAA AGAGCG

26

(2) INFORMATION FOR SEQ ID NO:56:

144

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 47 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TAYGARGACG AGGTGTCCTT CCTGGACGTA CACAGCCGCT AYCAYAC

47

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GCGGCCATCC GCATCTACGA CCTGGG

26

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CRTAGGCCGT CGGGCGRCAR CACGGGT

27

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GCGCCGAAGG CCCAGGTCGT AGATGCG

27

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

145

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CGCTACTGCG CAGGCGCGTG CGARGCGGC

29

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CGCCGACAGC TCTTGACGCG TRTGGA

27

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GAGCTGGGCC TGGGCTACGC GTCCGACGAG

30

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GCGACGCGTA CCATGAGGCG CTGGAAGGCA GCGGCCCTG

39

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

146

GACGGATCCG CATCACACGC ACGCGCACTC

30

## (2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GACCATATGC CGGGGGCTCG GCCTTGTGG

29

## (2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GACGGATCCG CATCACACGC ACGCGCACTC

30

## (2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CAGCGACGAC GCGTGCGCAA AGAGCG

26

## (2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TAGCGGCTGT GTACGTCCAG GAAGGACACC TCGT

34

## (2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:



147

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

AAAAATCGGG GGTGYGTCTT A

21

(2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CATGCCTGGC CTACYTTGTC A

21

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CTGGCGTCCC AMCAAGGGTC TTCG

24

(2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GCCAGTGGTG CCGTCGAGGC GGG

23

(2) INFORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

148

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGCCCAGGAT GAGGCGCTGG AAGG

24

(2) INFORMATION FOR SEQ ID NO:74:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CCTCTCCACT GCCTGAWATT CWACCCC

27

(2) INFORMATION FOR SEQ ID NO:75:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CCATGTGATT ATCGACCATT CGGC

24

(2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 134 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Ser Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu Arg Asn Arg  
1 5 10 15

Gln Ala Ala Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg  
20 25 30

Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu  
35 40 45

Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile  
50 55 60

Phe Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr Asp  
65 70 75 80

149

```

Lys Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val Ser Asp Lys
      85                      90                      95
Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp Asp Leu Ser
      100                    105                    110
Phe Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys His Ser Ala
      115                    120                    125
Lys Arg Cys Gly Cys Ile
      130

```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

[illegible]

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Ser Pro Asp Lys Gln Ala Ala Ala Leu Pro Arg Arg Glu Arg Asn Arg  
1 5 10 15

150

Gln Ala Ala Ala Ala Ser Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg  
                     20                    25                    30  
 Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu  
                     35                    40                    45  
 Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile  
                     50                    55                    60  
 Phe Arg Tyr Cys Ser Gly Ser Cys Glu Ala Ala Glu Thr Met Tyr Asp  
                     65                    70                    75                    80  
 Lys Ile Leu Lys Asn Leu Ser Arg Ser Arg Arg Leu Thr Ser Asp Lys  
                     85                    90                    95  
 Val Gly Gln Ala Cys Cys Arg Pro Val Ala Phe Asp Asp Asp Leu Ser  
                     100                    105                    110  
 Phe Leu Asp Asp Ser Leu Val Tyr His Ile Leu Arg Lys His Ser Ala  
                     115                    120                    125  
 Lys Arg Cys Gly Cys Ile  
                     130

## (2) INFORMATION FOR SEQ ID NO:79:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 89 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly  
 1                    5                    10                    15  
 Tyr Ala Ser Glu Glu Lys Val Ile Phe Arg Tyr Cys Ala Gly Ser Cys  
                     20                    25                    30  
 Pro Gln Glu Ala Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg  
                     35                    40                    45  
 Gly Arg Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr  
                     50                    55                    60  
 Ala Asp Val Thr Phe Leu Asp Asp Gln His His Trp Gln Gln Leu Pro  
                     65                    70                    75                    80  
 Gln Leu Ser Ala Ala Ala Cys Gly Cys  
                     85

## (2) INFORMATION FOR SEQ ID NO:80:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

151

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Ala Leu Ala Gly Ser Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala  
 1 5 10 15  
 Glu Leu Gly Leu Gly Tyr Ala Ser Glu Glu Lys Val Ile Phe Arg Tyr  
 20 25 30  
 Cys Ala Gly Ser Cys Pro Gln Glu Ala Arg Thr Gln His Ser Leu Val  
 35 40 45  
 Leu Ala Arg Leu Arg Gly Arg Gly Arg Ala His Gly Arg Pro Cys Cys  
 50 55 60  
 Gln Pro Thr Ser Tyr Ala Asp Val Thr Phe Leu Asp Asp Gln His His  
 65 70 75 80  
 Trp Gln Gln Leu Pro Gln Leu Ser Ala Ala Ala Cys Gly Cys Gly Gly  
 85 90 95

## (2) INFORMATION FOR SEQ ID NO:81:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 134 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Val Arg Ile Pro Gly Gly Leu Pro Thr Pro Gln Phe Leu Leu Ser Lys  
 1 5 10 15  
 Pro Ser Leu Cys Leu Thr Ile Leu Leu Tyr Leu Ala Leu Gly Asn Asn  
 20 25 30  
 His Val Arg Leu Pro Arg Ala Leu Ala Gly Ser Cys Arg Leu Trp Ser  
 35 40 45  
 Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly Tyr Ala Ser Glu Glu  
 50 55 60  
 Lys Val Ile Phe Arg Tyr Cys Ala Gly Ser Cys Pro Gln Glu Ala Arg  
 65 70 75 80  
 Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg Gly Arg Gly Arg Ala  
 85 90 95  
 His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr Ala Asp Val Thr Phe  
 100 105 110  
 Leu Asp Asp Gln His His Trp Gln Gln Leu Pro Gln Leu Ser Ala Ala  
 115 120 125  
 Ala Cys Gly Cys Gly Gly  
 130

## (2) INFORMATION FOR SEQ ID NO:82:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 89 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:

152

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

```

Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly
 1      5      10      15
Tyr Ala Ser Glu Glu Lys Ile Ile Phe Arg Tyr Cys Ala Gly Ser Cys
      20      25      30
Pro Gln Glu Val Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg
      35      40      45
Gly Gln Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr
      50      55      60
Ala Asp Val Thr Phe Leu Asp Asp His His His Trp Gln Gln Leu Pro
      65      70      75      80
Gln Leu Ser Ala Ala Ala Cys Gly Cys
      85

```

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 91 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

```

Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly
 1      5      10      15
Tyr Ala Ser Glu Glu Lys Ile Ile Phe Arg Tyr Cys Ala Gly Ser Cys
      20      25      30
Pro Gln Glu Val Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg
      35      40      45
Gly Gln Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr
      50      55      60
Ala Asp Val Thr Phe Leu Asp Asp His His His Trp Gln Gln Leu Pro
      65      70      75      80
Gln Leu Ser Ala Ala Ala Cys Gly Cys Gly Gly
      85      90

```

(2) INFORMATION FOR SEQ ID NO:84:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 267 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

TGCCGACTGT GGAGCCTGAC CCTACCACTG GCTGAGCTGG GCCTGGGCTA TGCCTCGGAG 60  
GAGAAGGTCA TCTTCCGATA CTGTGCTGGC AGCTGTCCCC AAGAGGCCCC TACCCAGCAC 120  
AGTCTGGTAC TGGCCCGGCT TCGAGGGCGG GGTGAGCCC ATGGCCGACC CTGCTGCCAG 180  
CCCACCAGCT ATGCTGATGT GACCTTCCTT GATGATCAGC ACCATTGGCA GCAGCTGCCT 240  
CAGCTCTCAG CTGCAGCTTG TGGCTGT 267

## (2) INFORMATION FOR SEQ ID NO:85:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 267 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TGCCGGCTGT GGAGCCTGAC CCTACCACTG GCTGAGCTTG GCCTGGGCTA TGCCTCAGAG 60  
GAGAAGATTA TCTTCCGATA CTGTGCTGGC AGCTGTCCCC AAGAGGTCCG TACCCAGCAC 120  
AGTCTGGTGC TGGCCCGTCT TCGAGGGCAG GGTGAGCTC ATGGCAGACC TTGCTGCCAG 180  
CCCACCAGCT ATGCTGATGT GACCTTCCTT GATGACCACC ACCATTGGCA GCAGCTGCCT 240  
CAGCTCTCAG CCGCAGCTTG TGGCTGT 267

## (2) INFORMATION FOR SEQ ID NO:86:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 273 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

TGCCGGCTGT GGAGCCTGAC CCTACCACTG GCTGAGCTTG GCCTGGGCTA TGCCTCAGAG 60  
GAGAAGATTA TCTTCCGATA CTGTGCTGGC AGCTGTCCCC AAGAGGTCCG TACCCAGCAC 120  
AGTCTGGTGC TGGCCCGTCT TCGAGGGCAG GGTGAGCTC ATGGCAGACC TTGCTGCCAG 180  
CCCACCAGCT ATGCTGATGT GACCTTCCTT GATGACCACC ACCATTGGCA GCAGCTGCCT 240  
CAGCTCTCAG CCGCAGCTTG TGGCTGTGGT GGC 273

## (2) INFORMATION FOR SEQ ID NO:87:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 94 amino acids

154

- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

```

Cys Val Leu Thr Ala Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly
 1           5           10           15
Tyr Glu Thr Lys Glu Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys
          20           25           30
Glu Ser Ala Glu Thr Met Tyr Asp Lys Ile Leu Lys Asn Leu Ser Arg
          35           40           45
Ser Arg Arg Leu Thr Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro
          50           55           60
Val Ala Phe Asp Asp Asp Leu Ser Phe Leu Asp Asp Asn Leu Val Tyr
65           70           75           80
His Ile Leu Arg Lys His Ser Ala Lys Arg Cys Gly Cys Ile
          85           90

```

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 95 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

```

Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu Leu Gly Leu Gly
 1           5           10           15
Tyr Thr Ser Asp Glu Thr Val Leu Phe Arg Tyr Cys Ala Gly Ala Cys
          20           25           30
Glu Ala Ala Ile Arg Ile Tyr Asp Leu Gly Leu Arg Arg Leu Arg Gln
          35           40           45
Arg Arg Arg Val Arg Arg Glu Arg Ala Arg Ala His Pro Cys Cys Arg
          50           55           60
Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp Val His Ser Arg
65           70           75           80
Tyr His Thr Leu Gln Glu Leu Ser Ala Arg Glu Cys Ala Cys Val
          85           90           95

```

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 91 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear



155

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly  
 1 5 10 15  
 Tyr Ala Ser Glu Glu Lys Val Ile Phe Arg Tyr Cys Ala Gly Ser Cys  
 20 25 30  
 Pro Gln Glu Ala Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg  
 35 40 45  
 Gly Arg Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr  
 50 55 60  
 Ala Asp Val Thr Phe Leu Asp Asp Gln His His Trp Gln Gln Leu Pro  
 65 70 75 80  
 Gln Leu Ser Ala Ala Ala Cys Gly Cys Gly Gly  
 85 90

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

TGCCTCAGAG GAGAAGATTA TC

22

(2) INFORMATION FOR SEQ ID NO:91:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 7 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Ala Ser Glu Glu Lys Ile Ile  
 1 5

(2) INFORMATION FOR SEQ ID NO:92:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

156

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Leu	Gly	Leu	Gly	Tyr	Glu	Thr	Lys	Glu	Glu	Leu	Ile	Phe	Arg	Tyr	Cys
1				5				10						15	

(2) INFORMATION FOR SEQ ID NO:93:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Leu	Gly	Leu	Gly	Tyr	Thr	Ser	Asp	Glu	Thr	Val	Leu	Phe	Arg	Tyr	Cys
1				5				10						15	

(2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Leu	Gly	Leu	Gly	Tyr	Ala	Ser	Glu	Glu	Lys	Ile	Ile	Phe	Arg	Tyr	Cys
1				5				10						15	

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

AGTCGGGGTT GGGGTATGCC TCA

23

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 base pairs
  - (B) TYPE: nucleic acid

157

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

TATGCCTCAG AGGAGAAGAT TATCTT

26

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 336 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

CCTCAGAGGA	GAAGATTATC	TTCCGATACT	GTGCTGGCAG	CTGTCCCAA	GAGGTCGGTA	60
CCCAGCACAG	TCTGGTGTCTG	GCCCGTCTTC	GAGGGCAGGG	TCGAGCTCAT	GGCAGACCTT	120
GCTGCCAGCC	CACCAGCTAT	GCTGATGTGA	CCTTCCTTGA	TGACCACCAC	CATTGGCAGC	180
AGCTGCCTCA	GCTCTCAGCC	GCAGCTTGTG	GCTGTGGTGG	CTGAAGGCGG	CCAGCCTGGT	240
CTCTCAGAAAT	CACAAGCAAG	AGGCAGCCTT	TGAAAGGCTC	AGGTGACGTT	ATTAGAAACT	300
TGCATAGGAG	AAGATTAAGA	AGAGAAAGGG	GACCTG			336

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

Ala Cys Cys Arg Pro Val Ala Phe Asp Asp Asp Leu Ser Phe Leu Asp  
1 5 10 15  
Asp

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

158

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Lys Asp  
1                      5                      10                      15

Val

(2) INFORMATION FOR SEQ ID NO:100:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 16 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

Pro Cys Cys Gln Pro Thr Ser Tyr Ala Asp Val Thr Phe Leu Asp Asp  
1                      5                      10                      15

(2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

TCATCAAGGA AGGTCACATC AGCATA

26

(2) INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

CCACCACAGC CACAAGCTGC GGSTGAGAGC TG

32

(2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid

159

(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Ala Leu Ala Gly Ser  
1 5

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Val Arg Ile Pro Gly Gly Leu Pro Thr Pro Gln Phe Leu Leu Ser Lys  
1 5 10 15  
Pro Ser Leu Cys Leu Thr Ile Leu Leu Tyr Leu Ala Leu Gly Asn Asn  
20 25 30  
His Val Arg Leu Pro Arg Ala Leu Ala Gly Ser  
35 40

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 544 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GAGGGACCTG GACGCCCCAT CAGGGTAAGA ATTCCTGGGG GCCTCCCGAC TCCCCAATTC	60
CTTCTCTCAA AGCCCTCACT TTGCCTTACA ATCCTACTCT ACCTTGCACT AGGTAACAAC	120
CATGTCCGTC TTCCAAGAGC CTTGGCTGGT TCATGCCGAC TGTGGAGCCT GACCCTACCA	180
GTGGCTGAGC TGGGCCTGGG CTATGCCTCG GAGGAGAAGG TCATCTTCCG ATACTGTGCT	240
GGCAGCTGTC CCCAAGAGGC CCGTACCCAG CACAGTCTGG TACTGGCCCG GCTTCGAGGG	300
CGGGGTCCGAG CCCATGGCCG ACCCTGCTGC CAGCCCACCA GCTATGCTGA TGTGACCTTC	360
CTTGATGATC AGCACCATTG GCAGCAGCTG CCTCAGCTCT CAGCTGCAGC TTGTGGCTGT	420
GGTGGCTGAA GGAGGCCAGT CTGGTGTCTC AGAATCACAA GCATGAGACA GGCTGGGCTT	480
TGAAAGGCTC AGGTGACATT ACTAGAAATT TGCATAGGTA AAGATAAGAA GGGAAAGGAC	540

160

CAGG

544

## (2) INFORMATION FOR SEQ ID NO:106:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 336 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

CCTCAGAGGA GAAGATTATC TTCCGATACT GTGCTGGCAG CTGTCCCCAA GAGGTCCGTA	60
CCCAGCACAG TCTGGTGCTG GCCCGTCTTC GAGGGCAGGG TCGAGCTCAT GGCAGACCTT	120
GCTGCCAGCC CACCAGCTAT GCTGATGTGA CCTTCCTTGA TGACCACCAC CATTGGCAGC	180
AGCTGCCTCA GCTCTCAGCC GCAGCTTG TGCTGTGGTG CTGAAGGCGG CCAGCCTGGT	240
CTCTCAGAA CACAAGCAAG AGGCAGCCTT TGAAAGGCTC AGGTGACGTT ATTAGAAACT	300
TGCATAGGAG AAGATTAAGA AGAGAAAGGG GACCTG	336

## (2) INFORMATION FOR SEQ ID NO:107:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 391 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

TGCCGGCTGT GGAGCCTGAC CCTACCACTG GCTGAGCTTG GCCTGGGCTA TGCCTCAGAG	60
GAGAAGATTA TCTTCCGATA CTGTGCTGGC AGCTGTCCCC AAGAGGTCCG TACCCAGCAC	120
AGTCTGGTGC TGGCCCGTCT TCGAGGGCAG GGTGAGCTC ATGGCAGACC TTGCTGCCAG	180
CCCACCAGCT ATGCTGATGT GACCTTCCTT GATGACCACC ACCATTGGCA GCAGCTGCCT	240
CAGCTCTCAG CCGCAGCTTG TGGCTGTGGT GGCTGAAGGC GGCCAGCCTG GTCTCTCAGA	300
ATCACAAGCA AGAGGCAGCC TTTGAAAGGC TCAGGTGACG TTATTAGAAA CTTGCATAGG	360
AGAAGATTAA GAAGAGAAAG GGGACCTGAT T	391

## (2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

161

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 2  
    (D) OTHER INFORMATION: /note= "SERINE, THREONINE, OR  
ALANINE"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 3  
    (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC  
ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Val Xaa Xaa Leu Gly Leu Gly Tyr  
1                    5

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 8 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS:  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 5  
    (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 7  
    (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Phe Arg Tyr Cys Xaa Gly Xaa Cys  
1                    5

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 8 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS:  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 2  
    (D) OTHER INFORMATION: /note= "ASPARTIC ACID, GLUTAMIC  
ACID OR NO AMINO ACID"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site  
    (B) LOCATION: 3  
    (D) OTHER INFORMATION: /note= "VALINE OR LEUCINE"

(ix) FEATURE:  
    (A) NAME/KEY: Modified-site

162

(B) LOCATION: 4

(D) OTHER INFORMATION: /note= "SERINE OR THREONINE"

## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 8

(D) OTHER INFORMATION: /note= "valine or aspartic acid"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Asp Xaa Xaa Xaa Phe Leu Asp Xaa  
 1 5

## (2) INFORMATION FOR SEQ ID NO:111:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 142 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Glu Gly Pro Gly Arg Pro Ile Arg Val Arg Ile Pro Gly Gly Leu Pro  
 1 5 10 15  
 Thr Pro Gln Phe Leu Leu Ser Lys Pro Ser Leu Cys Leu Thr Ile Leu  
 20 25 30  
 Leu Tyr Leu Ala Leu Gly Asn Asn His Val Arg Leu Pro Arg Ala Leu  
 35 40 45  
 Ala Gly Ser Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu  
 50 55 60  
 Gly Leu Gly Tyr Ala Ser Glu Glu Lys Val Ile Phe Arg Tyr Cys Ala  
 65 70 75 80  
 Gly Ser Cys Pro Gln Glu Ala Arg Thr Gln His Ser Leu Val Leu Ala  
 85 90 95  
 Arg Leu Arg Gly Arg Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro  
 100 105 110  
 Thr Ser Tyr Ala Asp Val Thr Phe Leu Asp Asp Gln His His Trp Gln  
 115 120 125  
 Gln Leu Pro Gln Leu Ser Ala Ala Ala Cys Gly Cys Gly Gly  
 130 135 140

## (2) INFORMATION FOR SEQ ID NO:112:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide



163

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Ala Leu Pro Gly Leu  
1 5

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "THREONINE, GLUTAMIC ACID OR LYSINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "VALINE, LEUCINE OR ISOLEUCINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "LEUCINE OR ISOLEUCINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Glu Xaa Xaa Xaa Phe Arg Tyr Cys Xaa Gly Xaa Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "ARGININE OR GLUTAMINE"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "THREONINE, VALINE OR

164

## ISOLEUCINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "ALANINE OR SERINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "TYROSINE OR PHENYLALANINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "GLUTAMIC ACID, ASPARTIC

ACID OR ALANINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 10
- (D) OTHER INFORMATION: /note= "GLUTAMIC ACID, ASPARTIC

ACID OR NO AMINO ACID"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 11
- (D) OTHER INFORMATION: /note= "VALINE OR LEUCINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /note= "SERINE OR THREONINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 16
- (D) OTHER INFORMATION: /note= "ASPARTIC ACID OR VALINE"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Asp Xaa Xaa Xaa Phe Leu Asp Xaa  
 1 5 10 15

## (2) INFORMATION FOR SEQ ID NO:115:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

GTNDGNGANY TGGGNYTGGG NTA

23

## (2) INFORMATION FOR SEQ ID NO:116:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

165

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GANBTNWCNT TYYTNGANG

19

(2) INFORMATION FOR SEQ ID NO:117:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

GANBTNWCNT TYYTNGANGW

20

(2) INFORMATION FOR SEQ ID NO:118:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

TTYMGNTAYT GYDSNGGND S NTG

23

(2) INFORMATION FOR SEQ ID NO:119:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

GTNDGNGANY TGGGNYTNGG

20

(2) INFORMATION FOR SEQ ID NO:120:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

166

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

GTNDGNGANY TGGGNYTGGG NTT

23

(2) INFORMATION FOR SEQ ID NO:121:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

WCNTCNARRA ANGWNAVNTC

20

(2) INFORMATION FOR SEQ ID NO:122:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

WCNTCNARRA ANGWNAVNT

19

(2) INFORMATION FOR SEQ ID NO:123:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

CANSHNCCNS HRCARTANCK RAA

23

(2) INFORMATION FOR SEQ ID NO:124:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

CANSHNCCNS HRCARTANCK RAANA

25

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 2  
(D) OTHER INFORMATION: /note= "THREONINE, SERINE OR  
ALANINE"

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 3  
(D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC  
ACID"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Val Xaa Xaa Leu Gly Leu Gly Tyr  
1 5

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 1  
(D) OTHER INFORMATION: /note= "ASPARTIC ACID OR GLUTAMIC  
ACID"

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 2  
(D) OTHER INFORMATION: /note= "VALINE OR LEUCINE"

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 3  
(D) OTHER INFORMATION: /note= "THREONINE OR SERINE"

(ix) FEATURE:  
(A) NAME/KEY: Modified-site  
(B) LOCATION: 6  
(D) OTHER INFORMATION: /note= "ASPARTIC ACID OR GLUTAMIC  
ACID"

168

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "ASPARTIC ACID OR VALINE"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Xaa Xaa Xaa Phe Leu Xaa Xaa  
1 5

## (2) INFORMATION FOR SEQ ID NO:127:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

Phe Arg Tyr Cys Xaa Gly Xaa Cys  
1 5

## (2) INFORMATION FOR SEQ ID NO:128:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "THREONINE, SERINE OR ALANINE"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "ASPARTIC ACID OR GLUTAMIC ACID"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

Val Xaa Xaa Leu Gly Leu Gly  
1 5

## (2) INFORMATION FOR SEQ ID NO:129:

169

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 8 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 2
  - (D) OTHER INFORMATION: /note= "THREONINE, SERINE OR ALANINE"
- (ix) FEATURE:
  - (A) NAME/KEY: Modified-site
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION: /note= "GLUTAMIC ACID OR ASPARTIC ACID"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:  
Val Xaa Xaa Leu Gly Leu Gly Phe  
1 5
- (2) INFORMATION FOR SEQ ID NO:130:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 9 amino acids
    - (B) TYPE: amino acid
    - (C) STRANDEDNESS:
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: protein
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 1
    - (D) OTHER INFORMATION: /note= "ISOLEUCINE OR LEUCINE"
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 6
    - (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"
  - (ix) FEATURE:
    - (A) NAME/KEY: Modified-site
    - (B) LOCATION: 8
    - (D) OTHER INFORMATION: /note= "SERINE OR ALANINE"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:  
Xaa Phe Arg Tyr Cys Xaa Gly Xaa Cys  
1 5
- (2) INFORMATION FOR SEQ ID NO:131:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 559 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

ATGGCTGCAG GAAGACTTCG GATCCTGTGT CTGCTGCTCC TGTCTTGCA CCCGAGCCTC	60
GGCTGGGTCC TTGATCTTCA AGAGGCTTCT GTGGCAGATA AGCTCTCATT TGGGAAGATG	120
GCAGAGACTA GAGGGACCTG GACGCCCCAT CAGGGTAAGA ATTCCTGGGG GCCTCCCGAC	180
TCCCCAATTC CTTCTCTCAA AGCCCTCACT TTGCCTTACA ATCCTACTCT ACCTTGCACT	240
AGGTAACAAC CATGTCCGTC TTCCAAGAGC CTTGGCTGGT TCATGCCGAC TGTGGAGCCT	300
GACCCTACCA GTGGCTGAGC TGGGCCTGGG CTATGCCTCG GAGGAGAAGG TCATCTTCCG	360
ATACTGTGCT GGCAGCTGTC CCCAAGAGGC CCGTACCCAG CACAGTCTGG TACTGGCCCCG	420
GCTTCGAGGG CGGGGTCGAG CCCATGGCCG ACCCTGCTGC CAGCCCACCA GCTATGCTGA	480
TGTGACCTTC CTTGATGATC AGCACCATTG GCAGCAGCTG CCTCAGCTCT CAGCTGCAGC	540
TTGTGGCTGT GGTGGCTGA	559

## (2) INFORMATION FOR SEQ ID NO:132:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 81 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Met	Ala	Ala	Gly	Arg	Leu	Arg	Ile	Leu	Cys	Leu	Leu	Leu	Ser	Leu		
1				5					10				15			
His	Pro	Ser	Leu	Gly	Trp	Val	Leu	Asp	Leu	Gln	Glu	Ala	Ser	Val	Ala	
			20					25					30			
Asp	Lys	Leu	Ser	Phe	Gly	Lys	Met	Ala	Glu	Thr	Arg	Gly	Thr	Trp	Thr	
			35				40					45				
Pro	His	Gln	Gly	Lys	Asn	Ser	Trp	Gly	Pro	Pro	Asp	Ser	Pro	Ile	Pro	
			50			55					60					
Ser	Leu	Lys	Ala	Leu	Thr	Leu	Pro	Tyr	Asn	Pro	Thr	Leu	Pro	Cys	Thr	
65				70					75					80		
Arg																

## (2) INFORMATION FOR SEQ ID NO:133:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 185 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Trp Leu Gln Glu Asp Phe Gly Ser Cys Val Cys Cys Ser Cys Pro Cys  
 1 5 10 15  
 Thr Arg Ala Ser Ala Gly Ser Leu Ile Phe Lys Arg Leu Leu Trp Gln  
 20 25 30  
 Ile Ser Ser His Leu Gly Arg Trp Gln Arg Leu Glu Gly Pro Gly Arg  
 35 40 45  
 Pro Ile Arg Val Arg Ile Pro Gly Gly Leu Pro Thr Pro Gln Phe Leu  
 50 55 60  
 Leu Ser Lys Pro Ser Leu Cys Leu Thr Ile Leu Leu Tyr Leu Ala Leu  
 65 70 75 80  
 Gly Asn Asn His Val Arg Leu Pro Arg Ala Leu Ala Gly Ser Cys Arg  
 85 90 95  
 Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly Tyr Ala  
 100 105 110  
 Ser Glu Glu Lys Val Ile Phe Arg Tyr Cys Ala Gly Ser Cys Pro Gln  
 115 120 125  
 Glu Ala Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg Gly Arg  
 130 135 140  
 Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr Ala Asp  
 145 150 155 160  
 Val Thr Phe Leu Asp Asp Gln His His Trp Gln Gln Leu Pro Gln Leu  
 165 170 175  
 Ser Ala Ala Ala Cys Gly Cys Gly Gly  
 180 185

(2) INFORMATION FOR SEQ ID NO:134:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 559 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

ATGGCTGCAG GAAGACTTCG GATCTTGTTT CTGCTGCTCC TGTCCTTGCA CCTGGGCCTT 60  
 GGCTGGGTCC TTGATCTTCA AGAGGCTCCT GCGGCAGATG AGCTCTCATC TGGGAAAATG 120  
 GCAGAGACTG GAAGGACCTG GAAGCCCCAT CAGGGTAAGA ATTCTTGGGG GCCTCCTAAC 180  
 TCTACAGTTC TTCCTCTCAA AGCCCTCACT TTGCCTCACA ATCCTATTCT ACCTTGCACT 240  
 AGGTAACAAC AATGTCCGCC TTCCAAGAGC CTTACCTGGT TTGTGCCGGC TGTGGAGCCT 300  
 GACCCTACCA GTGGCTGAGC TTGGCCTGGG CTATGCCTCA GAGGAGAAGA TTATCTTCCG 360  
 ATACTGTGCT GGCAGCTGTC CCAAGAGGT CCGTACCCAG CACAGTCTGG TGCTGGCCCCG 420  
 TCTTCGAGGG CAGGGTCGAG CTCATGGCAG ACCTTGCTGC CAGCCCACCA GCTATGCTGA 480  
 TGTGACCTTC CTTGATGACC ACCACCATTG GCAGCAGCTG CCTCAGCTCT CAGCCGCAGC 540

TTGTGGCTGT GGTGGCTGA

559

## (2) INFORMATION FOR SEQ ID NO:135:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 81 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

```

Met Ala Ala Gly Arg Leu Arg Ile Leu Phe Leu Leu Leu Leu Ser Leu
 1           5           10           15
His Leu Gly Leu Gly Trp Val Leu Asp Leu Gln Glu Ala Pro Ala Ala
      20           25           30
Asp Glu Leu Ser Ser Gly Lys Met Ala Glu Thr Gly Arg Thr Trp Lys
      35           40           45
Pro His Gln Gly Lys Asn Ser Trp Gly Pro Pro Asn Ser Thr Val Leu
      50           55           60
Pro Leu Lys Ala Leu Thr Leu Pro His Asn Pro Ile Leu Pro Cys Thr
      65           70           75           80
Arg

```

## (2) INFORMATION FOR SEQ ID NO:136:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 185 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

```

Trp Leu Gln Glu Asp Phe Gly Ser Cys Phe Cys Cys Ser Cys Pro Cys
 1           5           10           15
Thr Trp Ala Leu Ala Gly Ser Leu Ile Phe Lys Arg Leu Leu Arg Gln
      20           25           30
Met Ser Ser His Leu Gly Lys Trp Gln Arg Leu Glu Gly Pro Gly Ser
      35           40           45
Pro Ile Arg Val Arg Ile Leu Gly Gly Leu Leu Thr Leu Gln Phe Phe
      50           55           60
Leu Ser Lys Pro Ser Leu Cys Leu Thr Ile Leu Phe Tyr Leu Ala Leu
      65           70           75           80
Gly Asn Asn Asn Val Arg Leu Pro Arg Ala Leu Pro Gly Leu Cys Arg
      85           90           95
Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly Tyr Ala
      100          105          110

```

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Ser Glu Glu Lys Ile Ile Phe Arg Tyr Cys Ala Gly Ser Cys Pro Gln  
 115 120 125  
 Glu Val Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg Gly Gln  
 130 135 140  
 Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr Ala Asp  
 145 150 155 160  
 Val Thr Phe Leu Asp Asp His His His Trp Gln Gln Leu Pro Gln Leu  
 165 170 175  
 Ser Ala Ala Ala Cys Gly Cys Gly Gly  
 180 185

## (2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

AATCCCCAGG ACAGGCAGGG AAT

23

## (2) INFORMATION FOR SEQ ID NO:138:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 35 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

CGGTACCCAG ATCTTCAGCC ACCACAGCCA CAAGC

35

## (2) INFORMATION FOR SEQ ID NO:139:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 76 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

GGACTATCAT ATGGCCACC ACCACCACCA CCACCACCAC GACGACGACG ACAAGGCCTT  
 GGCTGGTTCA TGCCGA

60

76

## (2) INFORMATION FOR SEQ ID NO:140:

174

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

CGGTACCCAG ATCTTCAGCC ACCACAGCCA CAAGC

35

(2) INFORMATION FOR SEQ ID NO:141:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 96 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

Ala	Leu	Ala	Gly	Ser	Cys	Arg	Leu	Trp	Ser	Leu	Thr	Leu	Pro	Val	Ala
1				5				10					15		
Glu	Leu	Gly	Leu	Gly	Tyr	Ala	Ser	Glu	Lys	Val	Ile	Phe	Arg	Tyr	
		20					25					30			
Cys	Ala	Gly	Ser	Cys	Pro	Gln	Glu	Ala	Arg	Thr	Gln	His	Ser	Leu	Val
		35				40					45				
Leu	Ala	Arg	Leu	Arg	Gly	Arg	Gly	Arg	Ala	His	Gly	Arg	Pro	Cys	Cys
	50				55					60					
Arg	Pro	Thr	Ala	Tyr	Glu	Asp	Glu	Val	Ser	Phe	Leu	Asp	Val	His	Ser
65				70					75					80	
Arg	Tyr	His	Thr	Leu	Gln	Glu	Leu	Ser	Ala	Arg	Glu	Cys	Ala	Cys	Val
			85					90						95	

(2) INFORMATION FOR SEQ ID NO:142:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

TAATACGACT CACTATAGGG GAA

23

(2) INFORMATION FOR SEQ ID NO:143:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 49 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

175

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

TCGTCTTCGT AAGCAGTCGG ACGGCAGCAG GGTCGGCCAT GGGCTCGAC

49

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

TGCTGCCGTC CGACTGCTTA CGAAGACGA

29

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

GTTATGCTAG TTATTGCTCA GCGGT

25

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

Pro Gly Ala Arg Pro Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser  
 1 5 10 15

Glu Leu Gly Leu Gly Tyr Thr Ser Asp Glu Thr Val Leu Phe Arg Tyr  
 20 25 30

Cys Ala Gly Ala Cys Glu Ala Ala Ile Arg Ile Tyr Asp Leu Gly Leu  
 35 40 45

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Arg Arg Leu Arg Gln Arg Arg Arg Val Arg Arg Glu Arg Ala Arg Ala  
 50 55 60

His Pro Cys Cys Gln Pro Thr Ser Tyr Ala Asp Val Thr Phe Leu Asp  
 65 70 75 80

Asp Gln His His Trp Gln Gln Leu Pro Gln Leu Ser Ala Ala Ala Cys  
 85 90 95

Gly Cys Gly Gly  
 100

## (2) INFORMATION FOR SEQ ID NO:147:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 50 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

CACATCAGCA TAGCTGGTGG GCTGGCAGCA CGGGTGAGCA CGAGCACGTT

50

## (2) INFORMATION FOR SEQ ID NO:148:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

TGCTGCCAGC CCACCAGCTA TGCTG

25

## (2) INFORMATION FOR SEQ ID NO:149:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

CCTCGGAGGA GAAGGTCATC TTC

23

## (2) INFORMATION FOR SEQ ID NO:150:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 98 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single

177

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

```

Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly Trp
 1           5           10           15
Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu Gly
          20           25           30
Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val Leu
          35           40           45
Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys
          50           55           60
Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly Arg
65           70           75           80
Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys Lys
          85           90           95
Cys Ser

```

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 98 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

```

Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp
 1           5           10           15
Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala Gly
          20           25           30
Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val Leu
          35           40           45
Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys
          50           55           60
Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys
65           70           75           80
Thr Pro Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys
          85           90           95
Cys Ser

```

(2) INFORMATION FOR SEQ ID NO:152:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 98 amino acids

178

(B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp  
 1 5 10 15  
 Lys Trp Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly  
 20 25 30  
 Pro Cys Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu  
 35 40 45  
 Gly Leu Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys  
 50 55 60  
 Val Pro Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg  
 65 70 75 80  
 Thr Pro Lys Val Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys Lys  
 85 90 95  
 Cys Ser

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

Cys Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn  
 1 5 10 15  
 Asp Trp Ile Ile Ala Pro Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly  
 20 25 30  
 Glu Cys Pro Ser His Ile Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe  
 35 40 45  
 His Ser Thr Val Ile Asn His Tyr Arg Met Arg Gly His Ser Pro Phe  
 50 55 60  
 Ala Asn Leu Lys Ser Cys Cys Val Pro Thr Lys Leu Arg Pro Met Ser  
 65 70 75 80  
 Met Leu Tyr Tyr Asp Asp Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln  
 85 90 95  
 Asn Met Ile Val Glu Glu Cys Gly Cys Ser  
 100 105

(2) INFORMATION FOR SEQ ID NO:154:

(i) SEQUENCE CHARACTERISTICS:



179

- (A) LENGTH: 105 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

```

Cys Cys Arg Gln Gln Phe Phe Ile Asp Phe Arg Leu Ile Gly Trp Asn
 1           5           10           15
Asp Trp Ile Ile Ala Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly
          20           25           30
Ser Cys Pro Ala Tyr Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe
          35           40           45
His Thr Ala Val Val Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly
          50           55           60
Thr Val Asn Ser Cys Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met
65           70           75           80
Leu Tyr Phe Asp Asp Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn
          85           90           95
Met Ile Val Glu Glu Cys Gly Cys Ala
          100           105

```

(2) INFORMATION FOR SEQ ID NO:155:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 101 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

```

Cys Arg Arg Val Lys Phe Gln Val Asp Phe Asn Leu Ile Gly Trp Gly
 1           5           10           15
Ser Trp Ile Ile Tyr Pro Lys Gln Tyr Asn Ala Tyr Arg Cys Glu Gly
          20           25           30
Glu Cys Pro Asn Pro Val Gly Glu Glu Phe His Pro Thr Asn His Ala
          35           40           45
Tyr Ile Gln Ser Leu Leu Lys Arg Tyr Gln Pro His Arg Val Pro Ser
          50           55           60
Thr Cys Cys Ala Pro Val Lys Thr Lys Pro Leu Ser Met Leu Tyr Val
65           70           75           80
Asp Asn Gly Arg Val Leu Leu Glu His His Lys Asp Met Ile Val Glu
          85           90           95
Glu Cys Gly Cys Leu
          100

```

180

## (2) INFORMATION FOR SEQ ID NO:156:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
 1 5 10 15  
 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly  
 20 25 30  
 Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
 35 40 45  
 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala  
 50 55 60  
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
 65 70 75 80  
 Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu  
 85 90 95  
 Gly Cys Gly Cys Arg  
 100

## (2) INFORMATION FOR SEQ ID NO:157:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
 1 5 10 15  
 Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly  
 20 25 30  
 Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
 35 40 45  
 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala  
 50 55 60  
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
 65 70 75 80  
 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu  
 85 90 95

181

Gly Cys Gly Cys Arg  
100

## (2) INFORMATION FOR SEQ ID NO:158:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp  
1 5 10 15  
 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly  
20 25 30  
 Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala  
35 40 45  
 Val Val Gln Thr Leu Val Asn Asn Met Asn Pro Gly Lys Val Pro Lys  
50 55 60  
 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu  
65 70 75 80  
 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val  
85 90 95  
 Val Gly Cys Gly Cys Arg  
100

## (2) INFORMATION FOR SEQ ID NO:159:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
1 5 10 15  
 Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly  
20 25 30  
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
35 40 45  
 Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys  
50 55 60  
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
65 70 75 80

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
85 90 95

Arg Ser Cys Gly Cys His  
100

(2) INFORMATION FOR SEO ID NO:160:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 102 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

[illegible]

(2) INFORMATION FOR SEQ ID NO:161:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 102 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
1 5 10 15  
Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly  
20 25 30  
Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His Ala  
35 40 45  
Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro Lys  
50 55 60

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Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr Phe  
 65 70 75 80  
 Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
 85 90 95  
 Arg Ala Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:162:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu  
 1 5 10 15  
 Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly  
 20 25 30  
 Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys  
 50 55 60  
 Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr  
 65 70 75 80  
 Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val  
 85 90 95  
 Lys Ala Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:163:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp His  
 1 5 10 15  
 Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser Gly  
 20 25 30  
 Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro Lys  
 50 55 60

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Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr His  
 65 70 75 80  
 Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile Val  
 85 90 95  
 Lys Ser Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:164:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 103 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser  
 1 5 10 15  
 Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly  
 20 25 30  
 Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala  
 35 40 45  
 Thr Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile Pro  
 50 55 60  
 Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu Phe  
 65 70 75 80  
 Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met Thr  
 85 90 95  
 Val Glu Ser Cys Ala Cys Arg  
 100

## (2) INFORMATION FOR SEQ ID NO:165:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln  
 1 5 10 15  
 Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly  
 20 25 30  
 Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala  
 35 40 45

185

Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu  
 50 55 60  
 Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr  
 65 70 75 80  
 Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val  
 85 90 95  
 Asp Glu Cys Gly Cys Arg  
 100

## (2) INFORMATION FOR SEQ ID NO:166:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His  
 1 5 10 15  
 Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly  
 20 25 30  
 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala  
 35 40 45  
 Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly  
 50 55 60  
 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser  
 65 70 75 80  
 Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu  
 85 90 95  
 Asp Met Val Val Asp Glu Cys Gly Cys Arg  
 100 105

## (2) INFORMATION FOR SEQ ID NO:167:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

Cys His Arg His Gln Leu Phe Ile Asn Phe Gln Asp Leu Gly Trp His  
 1 5 10 15  
 Lys Trp Val Ile Ala Pro Lys Gly Phe Met Ala Asn Tyr Cys His Gly  
 20 25 30

[illegible]

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 103 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168;

Cys 1	Arg	Arg	Thr	Ser 5	Leu	His	Val	Asn	Phe 10	Lys	Glu	Ile	Gly	Trp 15	Asp
Ser	Trp	Ile	Ile 20	Ala	Pro	Lys	Asp	Tyr 25	Glu	Ala	Phe	Glu	Cys 30	Lys	Gly
Gly	Cys	Phe 35	Phe	Pro	Leu	Thr	Asp 40	Asn	Val	Thr	Pro	Thr 45	Lys	His	Ala
Ile	Val 50	Gln	Thr	Leu	Val 55	His	Leu	Gln	Asn	Pro	Lys 60	Lys	Ala	Ser	Lys
Ala 65	Cys	Cys	Val	Pro	Thr 70	Lys	Leu	Asp	Ala	Ile 75	Ser	Ile	Leu	Tyr	Lys 80
Asp	Asp	Ala	Gly	Val 85	Pro	Thr	Leu	Ile	Tyr 90	Asn	Tyr	Glu	Gly	Met 95	Lys
Val	Ala	Glu	Cys 100	Gly	Cys	Arg									

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 105 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

Cys His Arg Val Ala Leu Asn Ile Ser Phe Gln Glu Leu Gly Trp Glu  
1 5 10 15



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Arg Trp Ile Val Tyr Pro Pro Ser Phe Ile Phe His Tyr Cys His Gly  
                   20                  25                  30  
 Gly Cys Gly Leu His Ile Pro Pro Asn Leu Ser Leu Pro Val Pro Gly  
           35                  40                  45  
 Ala Pro Pro Thr Pro Ala Gln Pro Tyr Ser Leu Leu Pro Gly Ala Gln  
       50                  55                  60  
 Pro Cys Cys Ala Ala Leu Pro Gly Thr Met Arg Pro Leu His Val Arg  
   65                  70                  75                  80  
 Thr Thr Ser Asp Gly Gly Tyr Ser Phe Lys Tyr Glu Thr Val Pro Asn  
           85                  90                  95  
 Leu Leu Thr Gln His Cys Ala Cys Ile  
           100                  105

## (2) INFORMATION FOR SEQ ID NO:170:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 99 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

Cys Ala Leu Arg Glu Leu Ser Val Asp Leu Arg Ala Glu Arg Ser Val  
 1                  5                  10                  15  
 Leu Ile Pro Glu Thr Tyr Gln Ala Asn Asn Cys Gln Gly Ala Cys Gly  
           20                  25                  30  
 Trp Pro Gln Ser Asp Arg Asn Pro Arg Tyr Gly Asn His Val Val Leu  
       35                  40                  45  
 Leu Leu Lys Met Gln Ala Arg Gly Ala Thr Leu Ala Arg Pro Pro Cys  
   50                  55                  60  
 Cys Val Pro Thr Ala Tyr Thr Gly Lys Leu Leu Ile Ser Leu Ser Glu  
  65                  70                  75                  80  
 Glu Arg Ile Ser Ala His His Val Pro Asn Met Val Ala Thr Glu Cys  
           85                  90                  95  
 Gly Cys Arg

## (2) INFORMATION FOR SEQ ID NO:171:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

Cys Glu Leu His Asp Phe Ser Leu Ser Phe Ser Gln Leu Lys Trp Asp  
 1                  5                  10                  15

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```

Asn Trp Ile Val Ala Pro His Ser Tyr Asn Pro Ser Tyr Cys Lys Gly
    20                      25                      30
Asp Cys Pro Ser Ala Val Ser His Arg Tyr Gly Ser Pro Val His Thr
    35                      40                      45
Met Val Gln Asn Met Ile Tyr Glu Lys Leu Asp Pro Ser Val Pro Ser
    50                      55                      60
Pro Ser Cys Val Pro Gly Lys Tyr Ser Pro Leu Ser Val Leu Thr Ile
    65                      70                      75                      80
Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Met Ala
    85                      90                      95
Thr Ser Cys Thr Cys Arg
    100

```

## (2) INFORMATION FOR SEQ ID NO:172:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 94 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

```

Cys Val Leu Thr Ala Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly
 1                      5                      10                      15
Tyr Glu Thr Lys Glu Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys
    20                      25                      30
Asp Ala Ala Glu Thr Thr Tyr Asp Lys Ile Leu Lys Asn Leu Ser Arg
    35                      40                      45
Asn Arg Arg Leu Val Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro
    50                      55                      60
Ile Ala Phe Asp Asp Asp Leu Ser Phe Leu Asp Asp Asn Leu Val Tyr
    65                      70                      75                      80
His Ile Leu Arg Lys His Ser Ala Lys Arg Cys Gly Cys Ile
    85                      90

```

## (2) INFORMATION FOR SEQ ID NO:173:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 95 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

```

Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu Leu Gly Leu Gly
 1                      5                      10                      15

```

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Tyr Ala Ser Asp Glu Thr Val Leu Phe Arg Tyr Cys Ala Gly Ala Cys  
                     20                    25                    30  
 Glu Ala Ala Ala Arg Val Tyr Asp Leu Gly Leu Arg Arg Leu Arg Gln  
                     35                    40                    45  
 Arg Arg Arg Leu Arg Arg Glu Arg Val Arg Ala Gln Pro Cys Cys Arg  
                     50                    55                    60  
 Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp Ala His Ser Arg  
                     65                    70                    75                    80  
 Tyr His Thr Val His Glu Leu Ser Ala Arg Glu Cys Ala Cys Val  
                     85                    90                    95

## (2) INFORMATION FOR SEQ ID NO:174:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 291 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:

GCCTTGGCTG GTTCATGCCG ACTGTGGAGC CTGACCCTAC CAGTGGCTGA GCTGGGCCTG 60  
 GGCTATGCCT CGGAGGAGAA GGTCATCTTC CGATACTGTG CTGGCAGCTG TCCCCAAGAG 120  
 GCCCGTACCC AGCACAGTCT GGTACTGGCC CGGCTTCGAG GGCAGGGTCG AGCCCATGGC 180  
 CGACCTTGCT GCCAGCCAC CAGCTATGCT GATGTGACCT TCCTTGATGA TCAGCACCAT 240  
 TGGCAGCAGC TGCCTCAGCT CTCAGCTGCA GCTTGTGGCT GTGGTGGCTG A 291

## (2) INFORMATION FOR SEQ ID NO:175:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 405 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:

GTAAGAATTC CTGGGGGCCT CCCGACTCCC CAATTCCTTC TCTCAAAGCC CTCACCTTGC 60  
 CTTACAATCC TACTCTACCT TGCACTAGGT AACCAACCATG TCCGTCTTC AAGAGCCTTG 120  
 GCTGGTTCAT GCCGACTGTG GAGCCTGACC CTACCAAGTG CTGAGCTGGG CCTGGGCTAT 180  
 GCCTCGGAGG AGAAGGTCAT CTTCCGATAC TGTGCTGGCA GCTGTCCCCA AGAGGCCCGT 240  
 ACCCAGCACA GTCTGGTACT GGCCCGGCTT CGAGGGCGGG GTCGAGCCCA TGGCCGACCC 300  
 TGCTGCCAGC CCACCAGCTA TGCTGATGTG ACCTTCCTTG ATGATCAGCA CCATTGGCAG 360  
 CAGCTGCCTC AGCTCTCAGC TGCAGCTTGT GGCTGTGGTG GCTGA 405

## (2) INFORMATION FOR SEQ ID NO:176:

190

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 291 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:

GCCTTACCTG GTTTGTGCCG GCTGTGGAGC CTGACCCTAC CAGTGGCTGA GCTTGGCCTG	60
GGCTATGCCT CAGAGGAGAA GATTATCTTC CGATACTGTG CTGGCAGCTG TCCCCAAGAG	120
GTCCGTACCC AGCACAGTCT GGTGCTGGCC CGTCTTCGAG GGCAGGGTCG AGTCATGGC	180
AGACCTTGCT GCCAGCCCAC CAGCTATGCT GATGTGACCT TCCTTGATGA CCACCACCAT	240
TGGCAGCAGC TGCCTCAGCT CTCAGCCGCA GCTTGTGGCT GTGGTGGCTG A	291

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What is Claimed is:

1. An isolated and purified growth factor comprising persephin.
2. The isolated and purified growth factor of claim 1 comprising a polypeptide sequence which is at least about 85% sequence identity with SEQ ID NO:79 or SEQ ID NO:82.
3. The isolated and purified growth factor of claim 2 comprising a polypeptide sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:83 and conservatively substituted variants thereof.
4. The isolated and purified growth factor of Claim 1 in a pharmaceutically acceptable carrier.
5. The isolated and purified growth factor of claim 1 comprising a polypeptide containing a sequence of amino acids having at least about 65% sequence identity with SEQ ID NO:79 or SEQ ID NO:82 wherein the growth factor is from a non-mammalian species.
6. A method for obtaining a neurturin-persephin-GDNF family member growth factor comprising:
  - (a) isolating from a human genomic or cDNA library, a clone that hybridizes with a polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:76, SEQ ID NO:81, SEQ ID NO:83, and fragments thereof; and
  - (b) sequencing said clone.
7. A method for obtaining a neurturin-persephin-GDNF family member growth factor comprising:
  - (a) isolating from a human genomic or cDNA template, a clone that, upon application of a polymerase chain reaction method, produces an amplified polynucleotide having from about 30% to about 85% sequence identity to a region within a polynucleotide as set forth in a sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:76, and SEQ ID NO:81; and

(b) sequencing said clone.

8. The method according to claim 7 wherein the polymerase chain reaction method uses a degenerate primer obtained from a conserved region of any two of persephin, neurturin and GDNF and wherein the conserved region is as  
5 set forth in a sequence selected from the group consisting of SEQ ID NO:108, SEQ ID NO:113 and SEQ ID NO:114.

9. The method according to claim 8 wherein the conserved region degenerate primer has a sequence selected from the group consisting of SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119,  
5 SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, and SEQ ID NO:124.

10. An isolated and purified growth factor that is a neurturin-persephin-GDNF family member comprising a polypeptide having between about 30% and about 85% sequence identity with persephin, between about 30% and  
5 about 85% sequence identity with neurturin and between about 30% and about 85% sequence identity with GDNF.

11. The isolated and purified growth factor of claim 10 wherein said factor is comprised of a conserved region sequence of amino acids having at least a 62.5 percent sequence identity with SEQ ID NO:108 or at least  
5 a 62.5 percent sequence identity with SEQ ID NO:109 or at least a 50 percent sequence identity with SEQ ID NO:110.

12. The isolated and purified growth factor of claim 11 which is encoded by a nucleic acid identified and/or obtained by the polymerase chain reaction method utilizing a primer containing a nucleotide sequence  
5 selected from the group consisting of SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, and SEQ ID NO:124.

13. A pan-growth factor comprising a portion of the persephin polypeptide according to claim 1 and a

portion of at least one growth factor from the TGF-B superfamily other than persephin.

14. The pan-growth factor according to claim 13 wherein the growth factor other than persephin is neurturin and the pan-growth factor comprises SEQ ID NO:141 or SEQ ID NO:146.

15. An isolated and purified nucleic acid comprising a nucleotide sequence encoding a growth factor of any one of claims 1-3 and 10-14.

16. The isolated and purified nucleic acid which encodes a sequence selected from the group consisting of SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83 and conservatively substituted variants thereof.

17. The isolated and purified nucleic acid comprising SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:174, SEQ ID NO:175, and SEQ ID NO:176.

18. An isolated and purified nucleic acid sequence which hybridizes to a nucleotide sequence complementary to a nucleic acid sequence selected from the group consisting of:

- 5 (a) a nucleic acid according to claim 15;
- (b) a nucleic acid sequence which encodes persephin according to claim 1; and
- (c) a nucleic acid sequence which encodes a polypeptide which cross-reacts with persephin as defined in claim 1.

10 19. A vector comprising a recombinant DNA molecule comprising expression regulatory elements operably linked to a nucleic acid sequence according to claim 15.

20. A host cell transformed with the vector of claim 19.

21. The host cell according to claim 20 wherein said host cell is a mammalian cell, a bacterial cell or a baculovirus expression system.

22. A recombinant DNA method comprising:

(a) subcloning a DNA sequence encoding a growth factor as defined in claim 1 into an expression vector which comprises regulatory elements needed to express the DNA sequence;

5 (b) transforming a host cell with said expression vector;

(c) growing the host cell in a host cell culture; and

10 (d) harvesting the growth factor and/or the DNA from the host cell culture.

23. Isolated and purified antibodies which are capable of reacting with a growth factor as defined in claim 1 or an epitope thereof.

24. A method for preventing or treating cellular degeneration or insufficiency in an individual comprising administering to the individual a therapeutically effective amount of a growth factor as defined in any of  
5 claims 1-3 and 10-14 or a DNA molecule encoding said factor.

25. The method of claim 24 wherein the cellular degeneration or insufficiency is neuronal degeneration resulting from a condition selected from the group consisting of peripheral neuropathy, amyotrophic lateral  
5 sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Ischemic stroke, acute brain injury, acute spinal cord injury, nervous system tumors, multiple sclerosis, and infection.

26. The method of claim 24 wherein the cellular degeneration or insufficiency comprises hematopoietic cell degeneration or insufficiency resulting from a condition selected from the from the group consisting of  
5 eosinopenia, basopenia, lymphopenia, monocytopenia, neutropenia, anemias, thrombocytopenia, and stem-cell insufficiencies therefor.

27. The method of claim 24 wherein the cellular degeneration or insufficiency comprises cardiac muscle



degeneration or insufficiency resulting from cardiomyopathy or congestive heart failure.

28. A method for preventing or treating cellular degeneration or insufficiency in an individual comprising implanting into the individual cells that express a growth factor as defined in any of claims 1-3 and 10-14.

29. A method for detecting the presence of a growth factor in a sample from a patient comprising reacting antibodies according to claim 23 with a growth factor present in the sample and detecting a binding of  
5 the antibodies with the growth factor.

30. A kit for detecting the presence of a growth factor in a sample from a patient comprising antibodies according to claim 23 which are capable of detectably reacting with said growth factor packaged in a container.

31. A method for detecting the presence of a growth factor in a sample from a patient comprising detecting and/or quantitating the presence in the sample of mRNA encoding a growth factor of claims 1.

32. A method for detecting persephin gene alterations comprising detecting the presence of a non-intact persephin gene in a cell wherein presence of the non-intact gene indicates the presence of gene  
5 alterations.

33. A method for promoting the growth and/or differentiation of a cell in a culture medium comprising adding to the culture medium a growth factor as defined in claim 1.

34. An isolated and purified persephin antisense polynucleotide comprising a sequence complementary to a nucleic acid sequence of claim 15 and capable of hybridizing to a naturally-occurring DNA or mRNA  
5 polynucleotide sequence encoding persephin to prevent transcription and/or translation of an encoded persephin polypeptide.

35. A method for treating a disease condition mediated by expression of persephin by a population of cells comprising administering to said cells an inhibitory effective amount of the antisense
- 5 polynucleotide of claim 34.

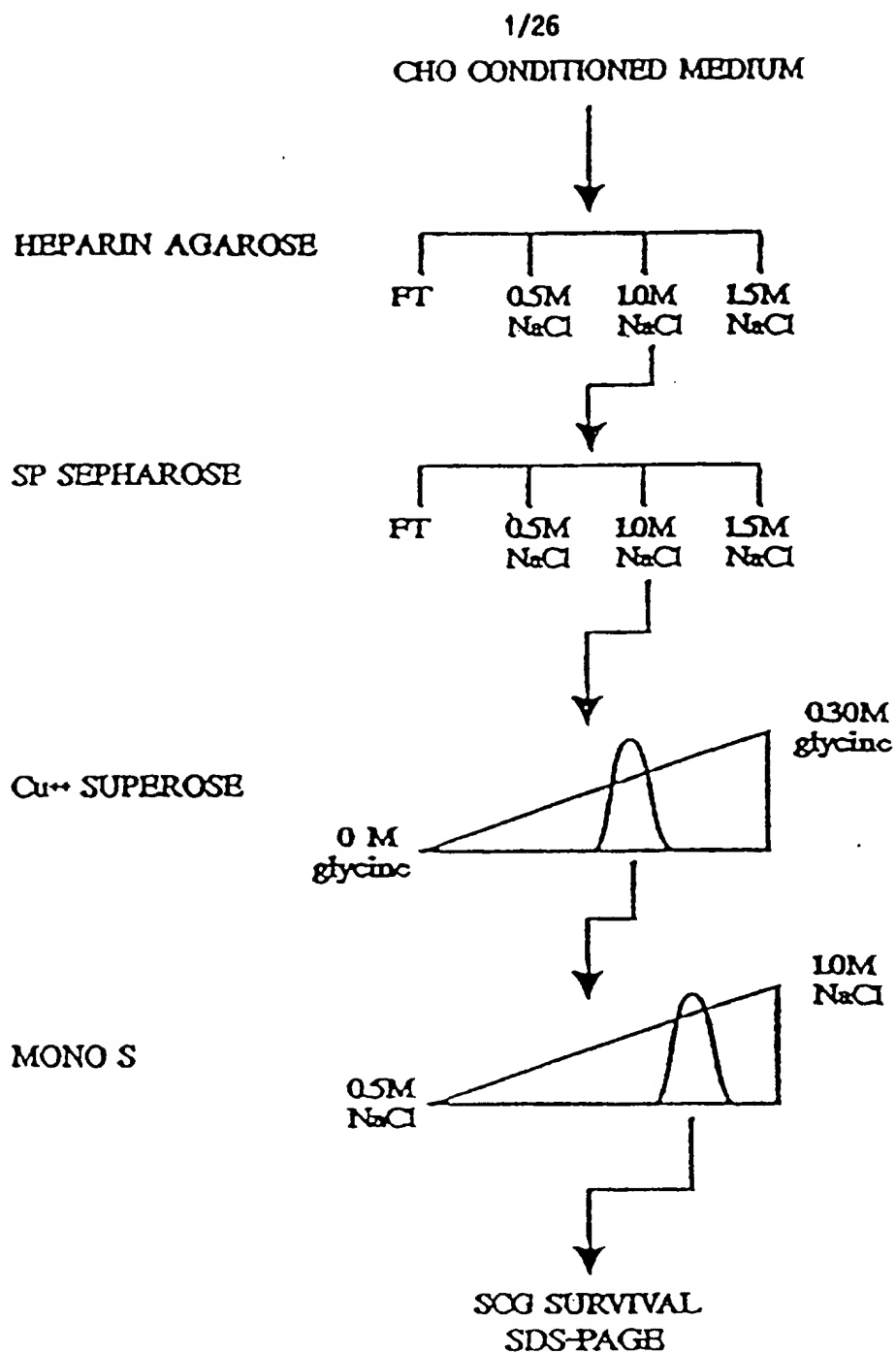


Figure 1

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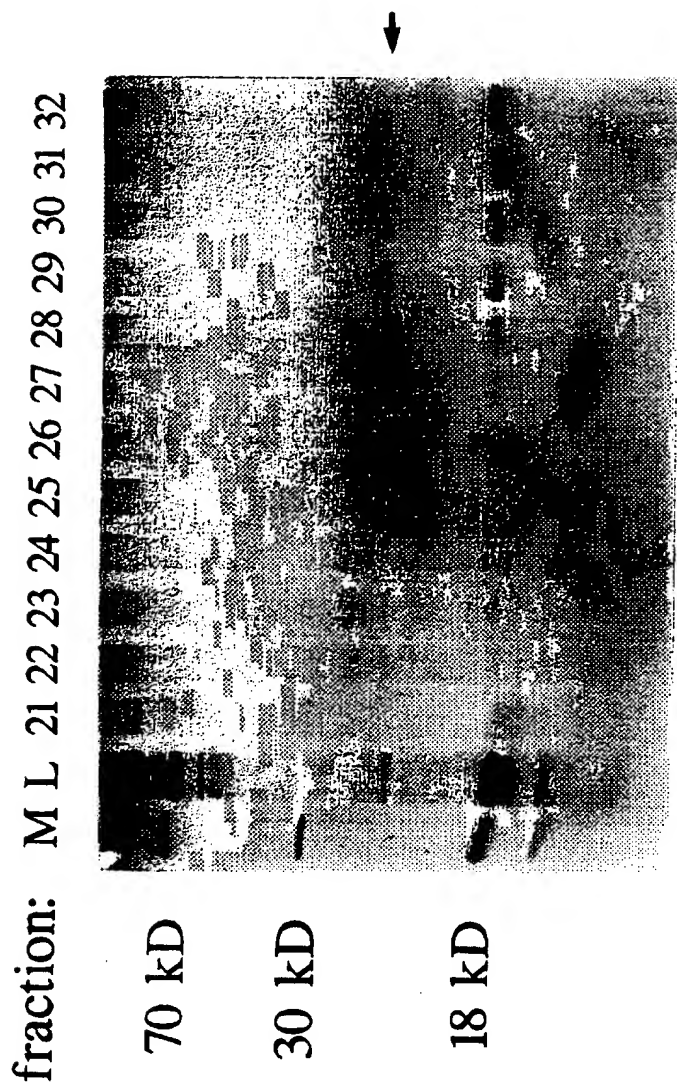


Figure 2a

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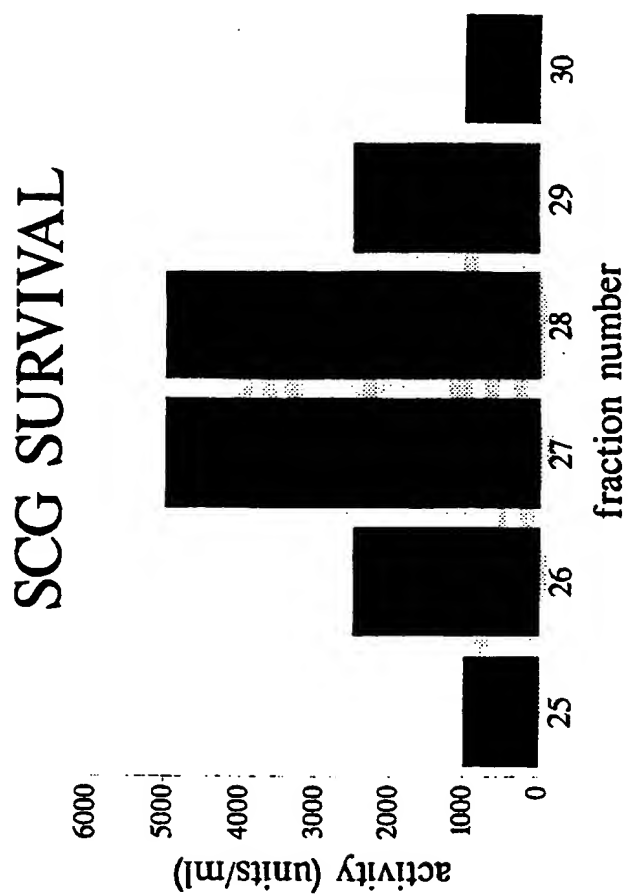


Figure 2b

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Figure 3a NGF

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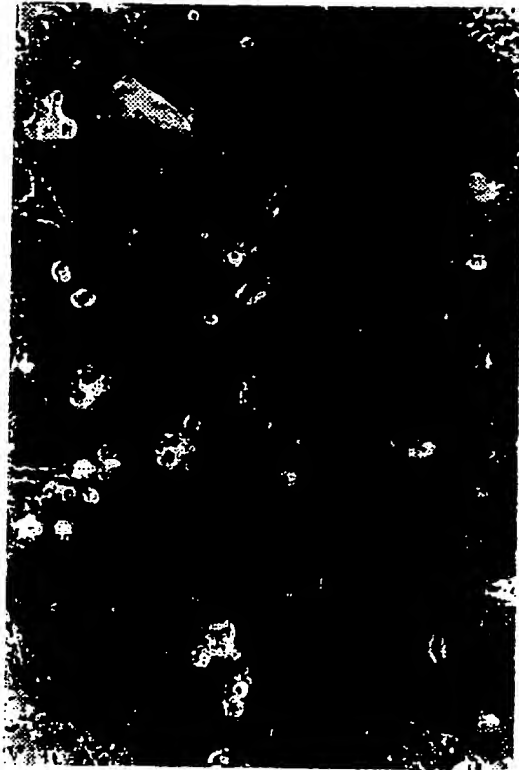


Figure 3b Anti-NGF

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Figure 3c Anti-NGF  
+  
Neurturin



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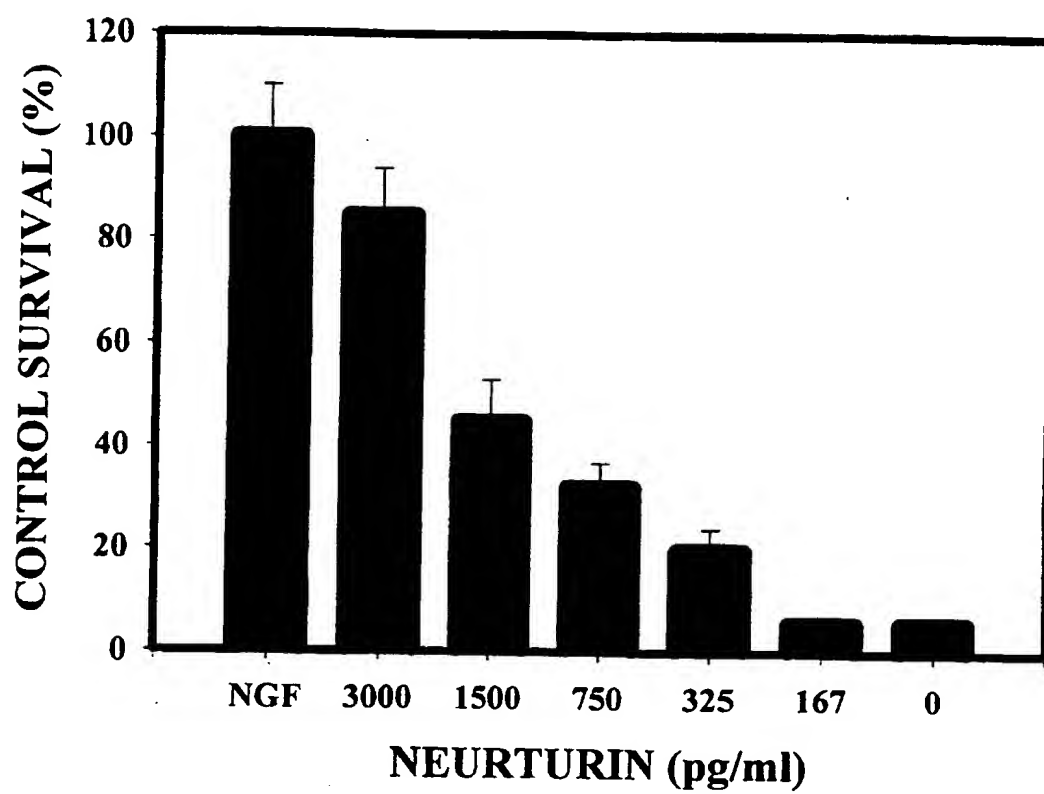


Figure 4

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1	S	P	D	K	Q	H	A	V	L	P	R	R	E	R	N	R	Q	A	A	A	A	N	P	E	N	S	R	G	K	G	hGDNF	
1	S	P	D	K	Q	A	A	A	L	P	R	R	E	R	N	R	Q	A	A	A	A	S	P	E	N	S	R	G	K	G	mGDNF	
1	S	P	D	K	Q	A	A	A	L	P	R	R	E	R	N	R	Q	A	A	A	A	S	P	E	N	S	R	G	K	G	rGDNF	
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	hNTN		
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	mNTN		
31	R	R	G	Q	R	G	K	N	R	G	C	V	L	T	A	I	H	L	N	V	T	D	L	G	L	G	Y	E	T	K	hGDNF	
31	R	R	G	Q	R	G	K	N	R	G	C	V	L	T	A	I	H	L	N	V	T	D	L	G	L	G	Y	E	T	K	mGDNF	
31	R	R	G	Q	R	G	K	N	R	G	C	V	L	T	A	I	H	L	N	V	T	D	L	G	L	G	Y	E	T	K	rGDNF	
1	-	-	-	-	A	R	L	G	A	R	P	C	G	L	R	E	L	E	V	R	V	S	E	L	G	L	G	Y	A	S	D	hNTN
1	-	-	-	-	-	P	G	A	R	P	C	G	L	R	E	L	E	V	R	V	S	E	L	G	L	G	Y	T	S	D	mNTN	
61	E	E	L	I	F	R	Y	C	S	G	S	C	E	S	A	A	E	T	T	Y	D	K	I	L	K	N	L	S	R	N	R	hGDNF
61	E	E	L	I	F	R	Y	C	S	G	S	C	E	S	A	A	E	T	M	Y	D	K	I	L	K	N	L	S	R	S	R	mGDNF
61	E	E	L	I	F	R	Y	C	S	G	S	C	E	S	A	A	E	T	M	Y	D	K	I	L	K	N	L	S	R	S	R	rGDNF
28	E	T	V	L	F	R	Y	C	A	G	A	C	E	A	A	A	R	V	Y	D	L	G	L	R	R	L	R	Q	R	R	R	hNTN
26	E	T	V	L	F	R	Y	C	A	G	A	C	E	A	A	I	R	I	Y	D	L	G	L	R	R	L	R	Q	R	R	R	mNTN
91	R	L	V	S	D	K	V	-	G	Q	A	C	C	R	P	I	A	F	D	D	D	L	S	F	L	D	D	N	L	V	hGDNF	
91	R	L	T	S	D	K	V	-	G	Q	A	C	C	R	P	V	A	F	D	D	D	L	S	F	L	D	D	N	L	V	mGDNF	
91	R	L	T	S	D	K	V	-	G	Q	A	C	C	R	P	V	A	F	D	D	D	L	S	F	L	D	D	S	L	V	rGDNF	
58	R	L	R	R	E	R	V	R	A	Q	P	C	C	R	P	T	A	Y	E	D	E	V	S	F	L	D	A	H	S	R	hNTN	
56	R	V	R	R	E	R	A	R	A	H	P	C	C	R	P	T	A	Y	E	D	E	V	S	F	L	D	V	H	S	R	mNTN	
120	Y	H	I	L	R	K	H	S	A	K	R	C	G	C	I	.	hGDNF															
120	Y	H	I	L	R	K	H	S	A	K	R	C	G	C	I	.	mGDNF															
120	Y	H	I	L	R	K	H	S	A	K	R	C	G	C	I	.	rGDNF															
88	Y	H	T	V	H	E	L	S	A	R	E	C	A	C	V	.	hNTN															
86	Y	H	T	L	Q	E	L	S	A	R	E	C	A	C	V	.	mNTN															

Figure 5

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RT-PCR Analysis of Neurturin and GDNF

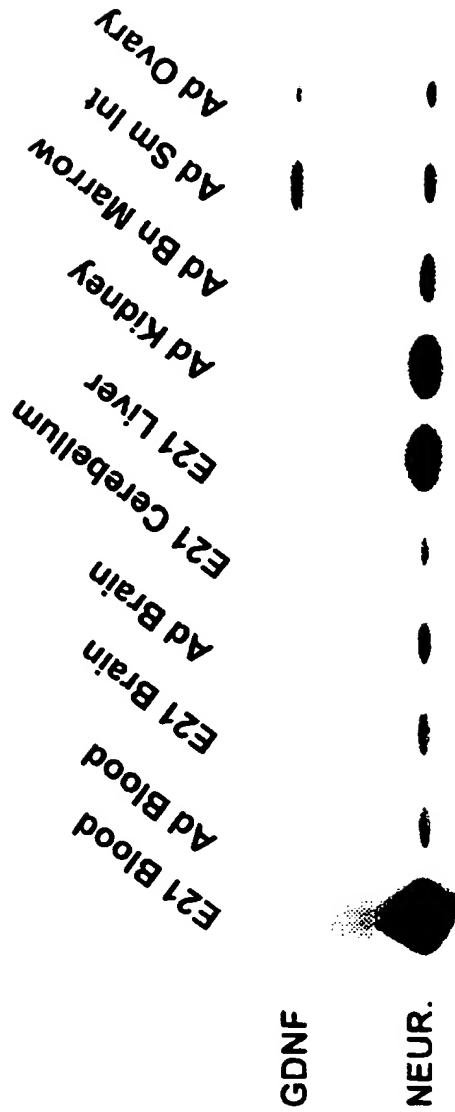


Figure 6

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ATGCAGCGCTGGAAGGCGGGCCCTTGGCCTCAGTGCTCTGCAGCTCCGTGCTGTCCATC 60  
Met Gln Arg Trp Lys Ala Ala Ala Leu Ala Ser Val Leu Cys Ser Ser Val Leu Ser Ile

TGGATGTGTCGAGAGGGCCTGCTTCTCAGCCACCGCCTCGGACCTGCGCTGGTCCCCTG 120  
Trp Met Cys Arg Glu Gly Leu Leu Leu Ser His Arg Leu Gly Pro Ala Leu Val Pro Leu

CACCGCCTGCCTCGAACCCTGGACGCCCCGGATTGCCCCGCCTGGCCCCAGT<sup>+</sup>ACCGTGCACTC 180  
His Arg Leu Pro Arg Thr Leu Asp Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu

CTGCAGGGGGCCCCGGATGCGATGGAGCTGCGCGAGCTGACGCCCCGGGCTGGGCGGGCCC 240  
Leu Gln Gly Ala Pro Asp Ala Met Glu Leu Arg Glu Leu Thr Pro Trp Ala Gly Arg Pro

CCAGGTCCGCGCCGTGGGGCGGGCCCCGGCGGGCGCGCGCGTGC<sup>+</sup>CGGTTGGGGGCG 300  
Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg Ala Arg Leu Gly Ala

CGGCCCTTGGGGCTGCGCGAGCTGGAGGTGCGCGTGAGCGAGCTGGGCCTGGGCTACGCG 360  
Arg Pro Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu Leu Gly Leu Gly Tyr Ala

TCCGACGAGACGGTGCTGTTCCGCTACTGCGCAGGCGCCTGCGAGGCTGCCGCGCGCGTC 420  
Ser Asp Glu Thr Val Leu Phe Arg Tyr Cys Ala Gly Ala Cys Glu Ala Ala Ala Arg Val

TACGACCTCGGGCTGCGACGACTGCGCCAGCGGCGGCGCCTGCGGGCGGGAGCGGGTGCGC 480  
Tyr Asp Leu Gly Leu Arg Arg Leu Arg Gln Arg Arg Arg Leu Arg Arg Glu Arg Val Arg

GCGCAGCCCTGCTGCCGCCCCGACGGCCTACGAGGACGAGGTGTCC<sup>+</sup>TTCTGGACGCGCAC 540  
Ala Gln Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp Ala His

AGCCGCTACCACACGGTGACGAGCTGTCGGCGCGCGAGTGCGCCTGCGTGTGA 594  
Ser Arg Tyr His Thr Val His Glu Leu Ser Ala Arg Glu Cys Ala Cys Val

Figure 7

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ATGAGGCGCTGGAAGGCAGCGGCCCTGGTGTGCTCATCTGCAGCTCCCTGCTATCTGTC 60  
Met Arg Arg Trp Lys Ala Ala Ala Leu Val Ser Leu Ile Cys Ser Ser Leu Leu Ser Val

TGGATGTGCCAGGAGGGTCTGCTCTTGGGCCACCGCCTGGGACCCGCGCTTGCCCCGCTA 120  
Trp Met Cys Gln Glu Gly Leu Leu Leu Gly His Arg Leu Gly Pro Ala Leu Ala Pro Leu

CGACGCCCTCCACGCACCCTGGACGCCCCGATCGCCCGCCTGGCCAGTATCGCGCTCTG 180  
Arg Arg Pro Pro Arg Thr Leu Asp Ala Arg Ile Ala Arg Leu Ala Gln Tyr Arg Ala Leu

CTCCAGGGCGCCCCGACGCGGTGGAGCTTCGAGAACTTTCTCCCTGGGCTGCCCGCATC 240  
Leu Gln Gly Ala Pro Asp Ala Val Glu Leu Arg Glu Leu Ser Pro Trp Ala Ala Arg Ile

CCGGGACCGCGCCGTCGAGCGGGTCCCCGGCGTCGGCGGGGCGCGGCCGGGGGCTCGGCCT 300  
Pro Gly Pro Arg Arg Arg Ala Gly Pro Arg Arg Arg Arg Ala Arg Pro Gly Ala Arg Pro

TGTGGGCTGCGCGAGCTCGAGGTGCGCGTGAGCGAGCTGGGCCTGGGCTACACGTCGGAT 360  
Cys Gly Leu Arg Glu Leu Glu Val Arg Val Ser Glu Leu Gly Leu Gly Tyr Thr Ser Asp

GAGACCGTGCTGTTCCGCTACTGCGCAGGCGCGTGCGAGGCGGCCATCCGCATCTACGAC 420  
Glu Thr Val Leu Phe Arg Tyr Cys Ala Gly Ala Cys Glu Ala Ala Ile Arg Ile Tyr Asp

CTGGGCCTTCGGCGCCTGCGCCAGCGGAGGCGCGTGCGCAGAGAGCGGGCGCGGGCGCAC 480  
Leu Gly Leu Arg Arg Leu Arg Gln Arg Arg Arg Val Arg Arg Glu Arg Ala Arg Ala His

CCGTGTTGTGCCCCGACGGCCTATGAGGACGAGGTGTCCTTCCTGGACGTGCACAGCCGC 540  
Pro Cys Cys Arg Pro Thr Ala Tyr Glu Asp Glu Val Ser Phe Leu Asp Val His Ser Arg

TACCACACGCTGCAAGAGCTGTCGGCGCGGGAGTGCGCGTGCGTGTA 588  
Tyr His Thr Leu Gln Glu Leu Ser Ala Arg Glu Cys Ala Cys Val •

Figure 8

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GGAGGGAGAGCGCGCGGTGGTTTCGTCCGTGTGCCCCGCGCCCGGCGC	-301
TCCTCGCGTGGCCCCGCGTCCTGAGCGCGCTCCAGCCTCCCACGCGCGCC	-251
ACCCCGGGGTTCAGTGAAGCCGCGAGCCCGGGGAAGACAGAGAAAGAGA	-201
GGCCAGGGGGGAACCCCATGGCCCGGCCGTGTCCCGCACCCCTGTGCGG	-151
TGGCCTCCTCCGGCACGGGGTCCCCGGGTGCGCTCCGGTCCCCGCGATCC	-101
GGATGGCGCACGCAGTGGCTGGGGCCGGGCCGGGCTCGGGTGGTCGGAGG	-51
AGTCACCACTGACCGGGTCATCTGGAGCCCGTGGCAGGCCGAGGCCCAGG	-1
ATGAGGCGCTGGAAGGCAGCGGCCCTGGTGTGCTCATCTGCAGCTCCCT	50
<u>GCTATCTGTCTGGATGTGCCAGGAGGGTCTGCTCTTGGGCCACCGCCTGG</u>	100
<u>GACCCGCGCTTGCCCCGCTACGACGCCCTCCACGCACCCTGGACGCCCCG</u>	150
<u>ATCGCCCGCCTGGCCCAGTATCGCGCTCTGCTCCAGGGCGCCCCGACGC</u>	200
<u>GGTGGAGCTTCGAGAACTTTCTCCCTGGGCTGCCCCGATCCCGGGACCGC</u>	250
<u>GCCGTGAGCGGGTCCCCGGCGTCGGCGGGCGGGCCGGGGGCTCGGCCT</u>	300
<u>TGTGGGCTGCGCGAGCTCGAGGTGCGCGTGAGCGAGCTGGGCCTGGGCTA</u>	350
<u>CACGTCGGATGAGACCGTGCTGTTCCGCTACTGCGCAGGCGCGTGCGAGG</u>	400
<u>CGGCCATCCGCATCTACGACCTGGGCCTTCGGCGCCTGCGCCAGCGGAGG</u>	450
<u>CGCGTGCGCAGAGAGCGGGCGCGGGCGCACCCGTGTGTGCGCCGACGGC</u>	500
<u>CTATGAGGACGAGGTGTCCTTCTGGACGTGCACAGCCGCTACCAACGC</u>	550
<u>TGCAAGAGCTGTCGGCGCGGGAGTGC</u> <u>GCGTGCGTGATGCTACCTCACG</u>	600
CCCCCGACCTGCGAAAGGGCCCTCCCTGCCGACCCTCGCTGAGAACTGA	650
CTTCACATAAAGTGTGGGAACTCCC	675

Figure 9

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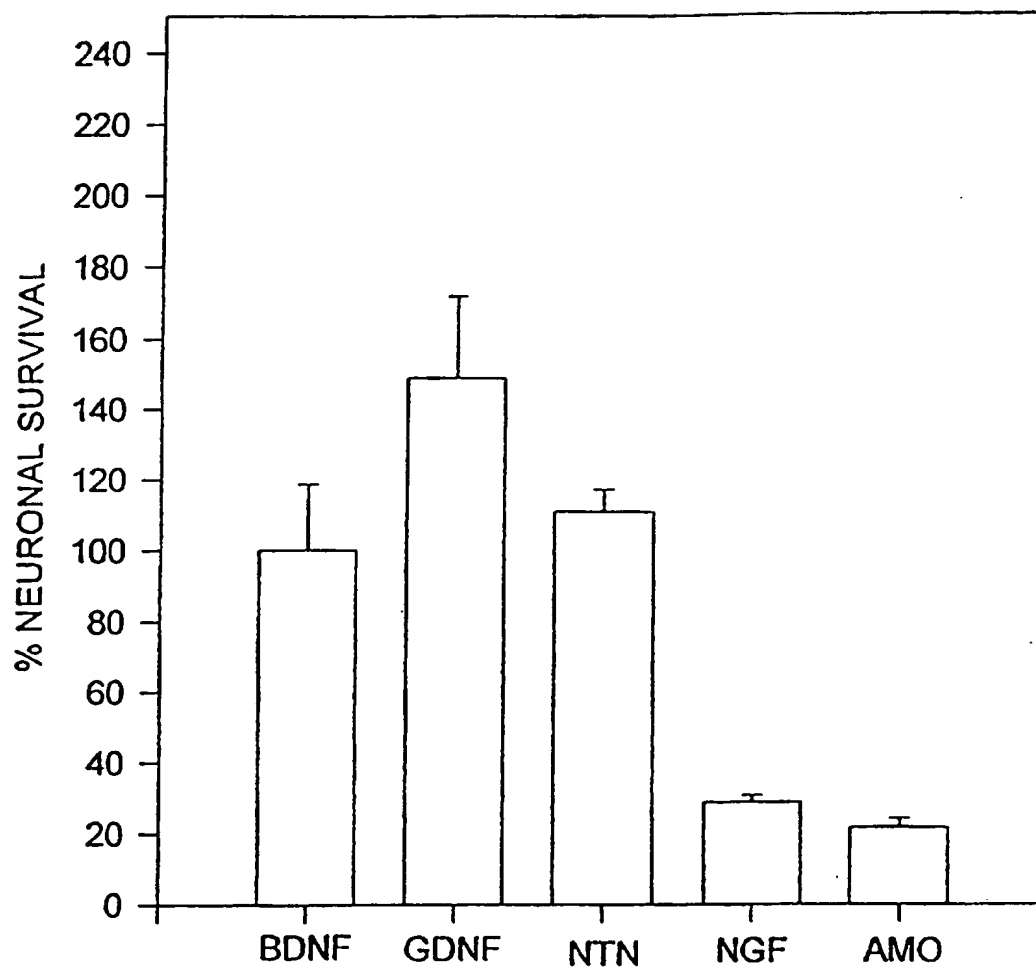


Figure 10

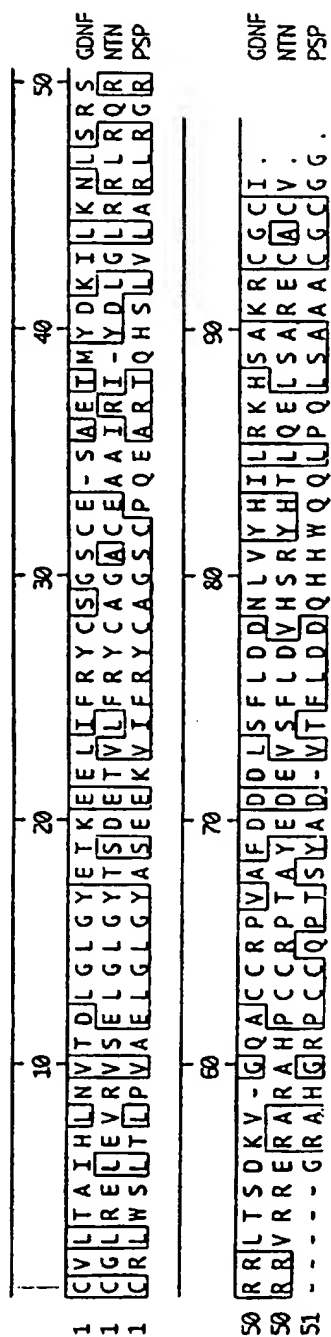
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GAGGGACCTGGACGCCCCATCAGGGTAAGAATTCCTGGGGGCCTCCCGACTCCCCAATTC	60
Glu Gly Pro Gly Arg Pro Ile Arg Val Arg Ile Pro Gly Gly Leu Pro Thr Pro Gln Phe	20
CTTCTCTCAAAGCCCTCACTTTGCCTTACAATCCTACTCTACCTTGCACTAGGTAACAAC	120
Leu Leu Ser Lys Pro Ser Leu Cys Leu Thr Ile Leu Leu Tyr Leu Ala Leu Gly Asn Asn	40
CATGTCCGTCTTCCAAGAGCCTTGGCTGGTTCATGCCGACTGTGGAGCCTGACCCTACCA	180
His Val Arg Leu Pro Arg Ala Leu Ala Gly Ser Cys Arg Leu Trp Ser Leu Thr Leu Pro	60
GTGGCTGAGCTGGGCCTGGGCTATGCCTCGGAGGAGAAGGTCATCTTCCGATACTGTGCT	240
Val Ala Glu Leu Gly Leu Gly Tyr Ala Ser Glu Glu Lys Val Ile Phe Arg Tyr Cys Ala	80
GGCAGCTGTCCCCAAGAGGCCCGTACCCAGCACAGTCTGGTACTGGCCCCGGCTTCGAGGG	300
Gly Ser Cys Pro Gln Glu Ala Arg Thr Gln His Ser Leu Val Leu Ala Arg Leu Arg Gly	100
CGGGGTCGAGCCCATGGCCGACCCTGCTGCCAGCCCACCAGCTATGCTGATGTGACCTTC	360
Arg Gly Arg Ala His Gly Arg Pro Cys Cys Gln Pro Thr Ser Tyr Ala Asp Val Thr Phe	120
CTTGATGATCAGCACCATTTGGCAGCAGCTGCCTCAGCTCTCAGCTGCAGCTTGTGGCTGT	420
Leu Asp Asp Gln His His Trp Gln Gln Leu Pro Gln Leu Ser Ala Ala Ala Cys Gly Cys	140
GGTGGCTGAAGGAGGCCAGTCTGGTGTCTCAGAATCACAAGCATGAGACAGGCTGGGCTT	480
Gly Gly	142
TGAAAGGCTCAGGTGACATTACTAGAAATTTGCATAGGTAAAGATAAGAAGGGAAAGGAC	540
CAGG	544

Figure 11



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CCTCAGAGGAGAAGATTATCTTCCGATACTGTGCTGGCAGCTGTCCCCAAGAGGTCCGTACC 62  
Ser Glu Glu Lys Ile Ile Phe Arg Tyr Cys Ala Gly Ser Cys Pro Gln Glu Val Arg Thr 20

CAGCACAGTCTGGTGCTGGCCCCGTCTTCGAGGGCAGGGTCGAGCTCATGGCAGACCTTGC 122  
Gln His Ser Leu Val Leu Ala Arg Leu Arg Gly Gln Gly Arg Ala His Gly Arg Pro Cys 40

TGCCAGCCCCACCAGCTATGCTGATGTGACCTTCCTTGATGACCACCACCATTGGCAGCAG 182  
Cys Gln Pro Thr Ser Tyr Ala Asp Val Thr Phe Leu Asp Asp His His His Trp Gln Gln 60

CTGCCTCAGCTCTCAGCCGCAGCTTGTGGCTGTGGTGGCTGAAGGCGGCCAGCCTGGTCT 242  
Leu Pro Gln Leu Ser Ala Ala Ala Cys Gly Cys Gly Gly 73

CTCAGAATCACAAGCAAGAGGCAGCCTTTGAAAGGCTCAGGTGACGTTATTAGAAACTTG 302

CATAGGAGAAGATTAAGAAGAGAAAAGGGGACCTG 336

Figure 13

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TGCCGGCTGTGGAGCCTGACCCTACCAGTGGCTGAGCTTGGCCTGGGCTATGCCTCAGAG	60
Cys Arg Leu Trp Ser Leu Thr Leu Pro Val Ala Glu Leu Gly Leu Gly Tyr Ala Ser Glu	20
GAGAAGATTATCTTCCGATACTGTGCTGGCAGCTGTCCCCAAGAGGTCCGTACCCAGCAC	120
Glu Lys Ile Ile Phe Arg Tyr Cys Ala Gly Ser Cys Pro Gln Glu Val Arg Thr Gln His	40
AGTCTGGTGCTGGCCCGTCTTCGAGGGGCAGGGTCGAGCTCATGGCAGACCTTGCTGCCAG	180
Ser Leu Val Leu Ala Arg Leu Arg Gly Gln Gly Arg Ala His Gly Arg Pro Cys Cys Gln	60
CCCACCAGCTATGCTGATGTGACCTTCCTTGATGACCACCACCATTGGCAGCAGCTGCCT	240
Pro Thr Ser Tyr Ala Asp Val Thr Phe Leu Asp Asp His His His Trp Gln Gln Leu Pro	80
CAGCTCTCAGCCGCAGCTTGTGGCTGTGGTGGCTGAAGGCGGCCAGCCTGGTCTCTCAGA	300
Gln Leu Ser Ala Ala Ala Cys Gly Cys Gly Gly	91
ATCACAAGCAAGAGGCAGCCTTTGAAAGGCTCAGGTGACGTTATTAGAAACTTGCATAGG	360
AGAAGATTAAGAAGAGAAAGGGGACCTGATT	391

Figure 14

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[illegible]

## Figure 15

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SEQ ID NO:	GROWTH FACTOR	SEQUENCE
150	TGFβ1	CCVRQLYIDFRKDLGWK-WTHEPKGYHANFCLGPCPYIWSLDT-----QYSKVLALYNQHNPGASAA-PCCV--PQALEPLPIVYVYGRKPKV--EQLSNMIVRSCKCS
151	TGFβ2	CCLRPLYIDFRKDLGWK-WTHEPKGYANFAGACPYLWSSDT-----QHSRVLSLYNTINPEASAS-PCCV--SQDLEPLTILYTGKTPKI--EQLSNMIVKSCKCS
152	TGFβ3	CCVRPLYIDFRQDLGWK-WHEPKGYANFCSGPCPYLRSADT-----THSTVLGLYNTLNPEASAS-PCCV--PQDLEPLTILYVGRTPKV--EQLSNMIVKSCKCS
153	INH8A	CKKQFFVSFK-DIGWNDWI IAPSGYHANYCEGECPSHIAG-TSGSSLSFHSITVINYRMRGHSFPANLKSCCV--PTKLRPMSMLYYDDGQNI I-KKDIQNMIVIEECGCS
154	INH8B	CCRQOFFIDFR-LIGWNDWI IAPTGYGNYCEGSCPAYLAG-VPGSASSFTAVNQYMRGLNP-GTVNSCCI--PTKLSTMSMLYFDDENYIV-KRDVPMIVIEECGCA
155	NODAL	CRRVKFQVDFN-LIGWSWI IYPKQYNAYRCEGECNPVGEEFHT-----NHAYIQSLLKRYQPHR-VPSTCCA--PVKTKPLSMLYVDNGR--VLLEHKKDMIVIEECGCL
156	BMP2	CKRHPLYVDFS-DVGWNDWI IAPGGYHAFYCHGECFPPLADHLNST-----NHAIVQTLVNSVNS-K-IPKACCV--PTELSAISMLYLDENEKVVK-K-NYQDMVVEGCGCR
157	BMP4	CRRHSLYVDFS-DVGWNDWI IAPGGYHAFYCHGECFPPLADHLNST-----NHAIVQTLVNSVNS-S-IPKACCV--PTELSAISMLYLDENEKVVK-K-NYQDMVVEGCGCR
158	DPP	CRRHSLYVDFS-DVGWDDWI IAPLGDAYYCHGKCPFLADHFNST-----NHAVVQTLVNNMNPVK-VPKPCCA--PTQLDSVAMLYLDQSTTVLK-NYQDMVVEGCGCR
159	BMP5	CKKHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
160	BMP6	CKKHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
161	BMP7	CKKHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
162	BMP8	CKKHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
163	60A	CRRHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
164	BMP3	CRRHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
165	VGL	CRRHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
166	GDF1	CRRHLYVSFR-DLGWODWI IAPEGYAAFYCDGECSPFLNAHMNAT-----NHAIVQTLVHLMPDH-VPKPCCA--PTKLNAISVLYFDDSSNVILK-KYRNMVVRSCGCH
167	GDF3	CHRHQLFINFO-DLGWODWI IAPKGFANYCQGCALPVALSGGGPPALNHAVALRALMHAAPGA-ADLPCCV--PTKMSPI SMLFYDNDNVILR-HYENMAVDECGCR
168	DORSLN	CRRHLYVSFR-DLGWODWI IAPKGFANYCQGCALPVALSGGGPPALNHAVALRALMHAAPGA-ADLPCCV--PTKMSPI SMLFYDNDNVILR-HYENMAVDECGCR
169	INHα	CHRHLYVSFR-DLGWODWI IAPKGFANYCQGCALPVALSGGGPPALNHAVALRALMHAAPGA-ADLPCCV--PTKMSPI SMLFYDNDNVILR-HYENMAVDECGCR
170	MIS	CHRHLYVSFR-DLGWODWI IAPKGFANYCQGCALPVALSGGGPPALNHAVALRALMHAAPGA-ADLPCCV--PTKMSPI SMLFYDNDNVILR-HYENMAVDECGCR
171	GDF9	CHRHLYVSFR-DLGWODWI IAPKGFANYCQGCALPVALSGGGPPALNHAVALRALMHAAPGA-ADLPCCV--PTKMSPI SMLFYDNDNVILR-HYENMAVDECGCR
172	GDNF	CVLTAHLNVT-DLGLG--YETKEELIFRYCSGSCD-AAETTYDKILKNLSRN-----RRLVSDKV-GQACCRPIAFD-DDLSFL-----DDNLVYHLRKHSKRCGCI
173	NTN	CGLRELEVRVS-ELGLG--YASDETIVLFYRCAGACE-AAARVYDLGLRRLRQR-----RRLRERVRAQPCCRPTAYE-DEVSLF-----DAHSRYHTVHEL SARECACV

SUBSTITUTE SHEET (RULE 26)

FIGURE 16

AGCACCATTTGGCAGCAGCTGCCTCAGCTCTCAGCTGCAGCTGTGGCTGTGGTGGCTGA 559

Q H H W Q Q L P Q L S A A C G C G G . 185

Figure 17

Figure 18

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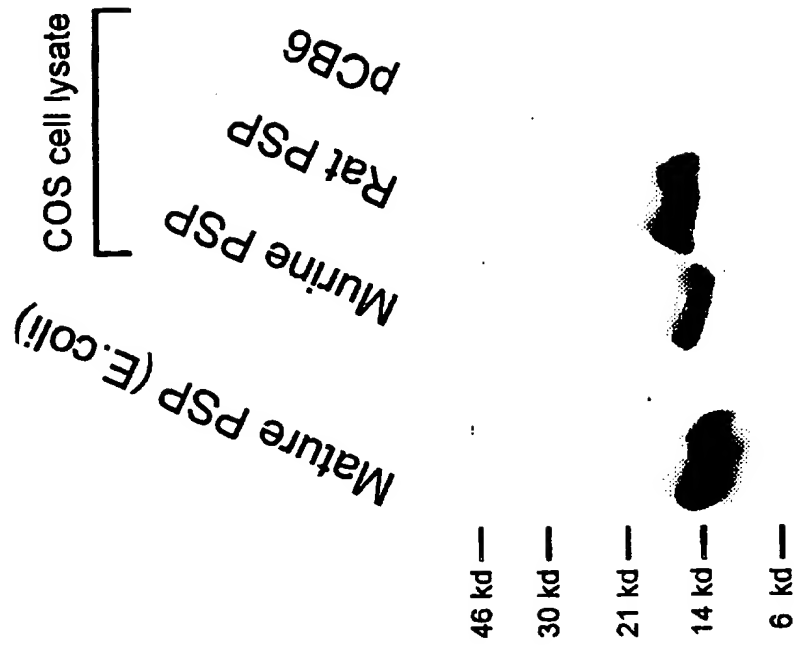


Figure 19



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**FIGURE 20A****PSP/NTN (SEQ ID NO:137)**

ALAGSCLWSLTLPVAELGLGYASEEKVIFRYCAGSCPQEARTQHSLVLA	50
↓	
RLRGRGRAHGRPCCRPTAYEDEVSFLDVHSRYHTLQELSARECACV	96

**FIGURE 20B****NTN/PSP (SEQ ID NO:142)**

PGARPCGLRELEVRVSELGLGYTSDETTLFRYCAGACEAAIRIYDLGLRR	50
↓	
LRQRRRVRRERARAHPCCPQTSYADVTFLLDDQHHWQQLPQLSAAACGCGG	100

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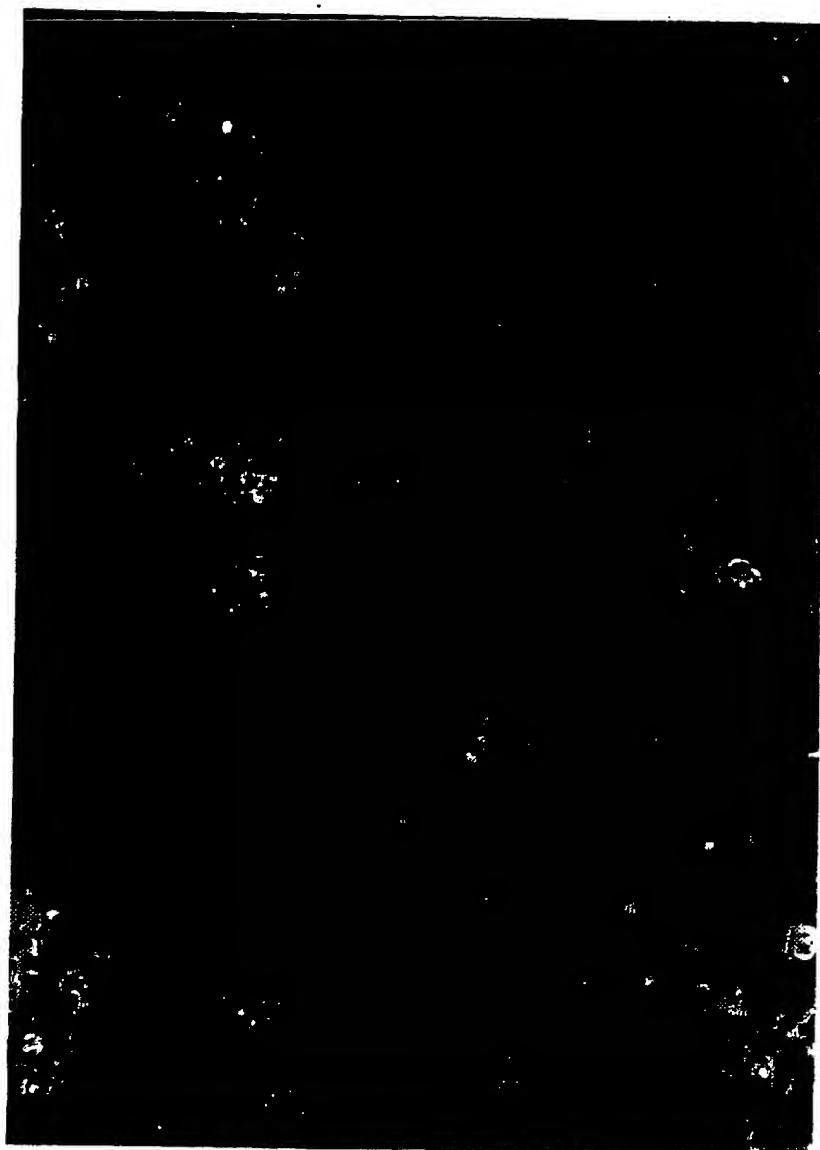


Figure 21a

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Figure 21b

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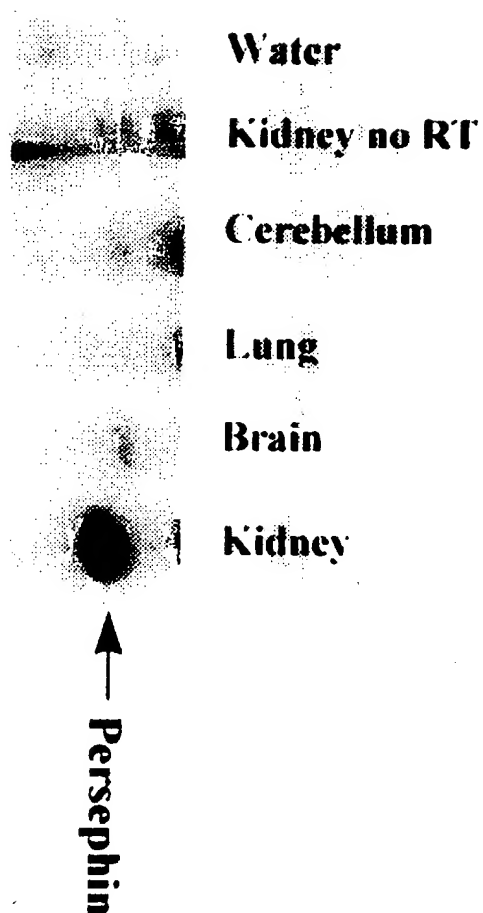


Figure 22

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03461

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399, 387.1; 435/69.4, 69.7, 325, 252.3, 320.1, 6, 7.1, 91.2; 536/23.51, 24.1, 24.31; 514/12, 44; 424/93.21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, IntelliGenetics

search terms: persephin, GDNF, neurturin, glia# derived neurotrophic factor#

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIN et al. GDNF: A Glial Cell Line-Derived Neurotrophic Factor for Midbrain Dopaminergic Neurons. Science. 21 May 1993. Vol. 260. pages 1130-1132.	2-3, 5-12, 15-22, 34
X	WATABE et al. Spontaneously Immortalized Adult Mouse Schwann Cells Secrete Autocrine and Paracrine Growth-Promoting Activities. Journal of Neuroscience Research. 01 June 1995. Vol 41. No. 2. pages 279-290.	2-3, 5-12, 15-22, 34
X	SPRINGER et al. CDNA Sequence and Differential mRNA Regulation of Two Forms of Glial Cell Line-Derived Neurotrophic Factor in Schwann Cells and Rat Skeletal Muscle. Experimental Neurology. January 1995. Vol. 131. No. 1. pages 47-52.	2-3, 5-12, 15-22, 34

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

03 JULY 1997

Date of mailing of the international search report

26 AUG 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
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Authorized officer

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/03461

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/17203 A1 (THE UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY) 29 June 1995, see entire document.	2-3, 5-12, 15-22, 24-25, 34
A	JACKOWSKI, A. Neural Injury Repair: Hope for the Future as Barriers to Effective CNS Regeneration Become Clearer. British Journal of Neurosurgery. 1995. Vol. 9. pages 303-317.	1-35
A	STULL et al. Antigene, Ribozyme and Aptamer Nucleic Acid Drugs: Progress and Prospects. Pharmaceutical Research. 1995. Vol. 12. No. 4. pages 465-483.	24-28, 34-35
A	SLOAN et al. The Immune Response to Intracerebral Neural Grafts. TINS. 1991. Vol. 14. No 8. pages 341-346.	28
X,P	KOTZBAUER et al. Neurturin, a Relative of Glial-Cell-Line-Derived Neurotrophic Factor. Nature. 05 December 1996. Vol. 384. No. 6608. pages 467-470.	2-3, 5-12, 15-22, 34

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03461

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/03461

## A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C07K 14/475, 16/26; C12N 15/18, 15/85, 15/63, 1/21; C07H 21/00; A61K 38/18, 48/00; C12Q 1/68; G01N 33/53; C12P 19/34

## A. CLASSIFICATION OF SUBJECT MATTER: US CL :

530/399, 387.1; 435/69.4, 69.7, 325, 252.3, 320.1, 6, 7.1, 91.2; 536/23.51, 24.1, 24.31; 514/12, 44; 424/93.21

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-5 and 10-22, drawn to growth factors comprising persephin, or pan-growth factors, pharmaceutical compositions thereof, vectors, host cells, and method of producing these growth factors recombinantly.

Group II, claim(s) 6-9, drawn to a method of obtaining a neurturin-persephin-GDNF family member growth factor using hybridization protocols, or PCR.

Group III, claim(s) 23, drawn to antibodies of growth factors that comprise persephin.

Group IV, claim(s) 24-27 and 33, drawn to methods of preventing/treating cellular degeneration comprising administering to a patient a therapeutically effective amount of a growth factor comprising persephin, and *in vitro* methods of promoting growth.

Group V, claim(s) 24-28, drawn to gene/cell therapy methods of preventing/treating cellular degeneration comprising administering to a patient a DNA molecule encoding a growth factor comprising persephin, or implanting cells expressing the growth factor.

Group VI, claim(s) 29-30, drawn to kits and methods of detecting a growth factor comprising persephin in a sample comprising use of antibodies.

Group VII, claim(s) 31-32, drawn to methods of detecting a growth factor comprising persephin in a sample comprising detecting nucleic acids.

Group VIII, claims 34-35, drawn to antisense persephin polynucleotides and gene therapy methods using the antisense molecule to inhibit persephin expression in a population of cells.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I is directed to growth factors comprising persephin, or pan-growth factors, pharmaceutical compositions thereof, vectors, host cells, and a method of producing these growth factors recombinantly, which is the first appearing product, method of use and method of making the growth factors. The special technical feature is the persephin growth factor protein. Groups II and IV-VIII are drawn to methods having different goals, method steps and starting materials, which do not require each other for their practice and do not share the same or a corresponding technical feature. Groups I, III, VI and VIII are drawn to structurally different products, which do not require each other for their practice and do not share the same or a corresponding technical feature. Note that PCT Rule 13 does not provide for multiple products or methods within a single application. Since the special technical feature of the Group I invention is not present in the Group II-VIII claims, and the special technical features of the Group II-VIII inventions are not present in the Group I claims, unity of invention is lacking.